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Vitamin A (Retinol) And Postoperative Colorectal Cancer

by

Una Man-shu Chan

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES & RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF Master of Science

IN

Nutrition

Faculty Of Home Economics

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES & RESEARCH

The undersigned certify that they have read, and recommend to the Faculty Of Graduate Studies & Research, for acceptance, a thesis entitled Vitamin A (Retinol) And Postoperative Colorectal Cancer submitted by Una Man-shu Chan in partial fulfilment of the requirements for the degree of Master of Science in Nutrition.

Abstract

In order to determine whether low plasma levels of vitamin A were related to increased risk of cancer recurrence, plasma vitamin A was measured in 103 patients (54 males, 49 females) who had had colorectal cancer surgically removed. According to the modification of the Dukes' classification, 66 had B2 tumors (i.e. tumor with no nodal involvement); 37 had C tumors (i.e tumor with lymph-node metastases). These patients were part of the Cross Cancer Institute Adjuvant GI Cohorts who were on the control arms receiving no further treatment, and had been followed from 5 months to 6 years after surgery. At the time of blood sample collection, they were believed to be free of neoplastic disease. Their results were compared with 65 (34 males, 31 females) apparently healthy control subjects who were Red Cross blood donors from various locations in Northern Alberta.

In addition to vitamin A, plasma RBP, cholesterol and cortisol and proteins which are related in the metabolism of this vitamin were also measured.

Results indicated that the plasma concentrations of vitamin A were sigificantly lower in both groups of patients when compared with the healthy subjects ($p<0.001$). RBP levels appeared to be significantly lower only in Dukes' C patients. Plasma cholesterol, cortisol, the albumin and globulin ratio, however, remained unaffected. These findings appeared to be persistent during the follow-up

study when a second blood sample was collected less than 1 to 4 months later from 40 patients. Furthermore, plasma vitamin A , in conjunction with RBP, was found to be even lower in those who subsequently had cancer recurrence than in those who remained free of apparent cancer. Moreover, the two patients who had died of the disease during the study exhibited severely low values of both plasma vitamin A and RBP. The significance of these results will be discussed.

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Abbreviations

BCG = Bacille Calmette Guerin

BP = benzopyrene

CRABP = cellular retinoic acid-binding protein

CRBP = cellular retinol-binding protein

dl = deciliter

DMBA = dimethylbenz[a]anthracene

DNA = deoxyribonucleic acid

5-Fu = 5-flurouracil

GI = gastrointestinal

mcg = microgram

mcl = microliter

MeCCNU = 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (semustine)

mg = milligram

ml = milliliter

mm = millimeter

nm = nanometer

RBP = retinol-binding protein

RR = relative risk

SEM = standard error of the mean

3-MC = 3-methylcholanthrene

WWCCI = W.W. Cross Cancer Institute

Chapter 1

INTRODUCTION

The crucial role of vitamin A (retinol) in the control of cellular differentiation in epithelial tissues has long been recognized (Moore, 1967). Vitamin A and its analogs (retinoids) have been under study in recent years both as inhibitors of carcinogenesis and as potential anti-tumor agents. Their ability to inhibit chemical carcinogen induced epithelial cancer of the skin, lung, bladder, and breast in experimental animals is well documented (Sporn et al., 1976; Sporn, 1977).

Subnormal levels of plasma vitamin A have also been implicated as a possible cause of epithelial cancers of the lung, oropharynx, bladder and gastrointestinal tract in humans by both epidemiological and biochemical studies. Most of these studies of the hypothesis have relied on results in blood samples after the diagnosis of cancer. Although recent prospective studies have indicated that low plasma retinol levels are associated with increased risk of developing cancer (Wald et al., 1980; Kark et al., 1981, Haines et al., 1982), evidence for a relationship between vitamin A and subsequent cancer recurrence in patients who had undergone curative surgery is virtually non-existent. This is the underlying motivation towards the initiation of the present study.

1.1 Background Information

1.1.1 Functions Of Vitamin A

Vitamin A occurs physiologically as the alcohol (retinol), the aldehyde (retinaldehyde), the acid (retinoic acid) and the ester (retinyl ester). Sporn (1976) has coined the term "retinoids" to describe all moieties of vitamin A molecules.

Vitamin A has a number of important functions in the body. It is necessary for vision, bone development, reproduction, and normal differentiation of epithelial tissues. These functions are mediated by the different forms of the molecule. The biologically active form in mammalian tissue is all-trans retinol which can be oxidized to the aldehyde, retinal, which is involved in the visual cycle (Wald, 1968). This is a reversible reaction, as retinal can also be converted by the body to retinol. Oxidation of retinol also produces irreversibly retinoic acid which is capable of promoting growth and differentiation of epithelial tissues, but it is not active in vision (Dowling & Wald, 1960) and reproduction (Pawson, 1981; Thompson et al., 1964).

1.1.2 Vitamin A and Epithelial Structures

Of all the functions mentioned above, the most profound effect of vitamin A is its ability in controlling the normal differentiation and maintenance of epithelial tissues (De

Luca et al., 1972). The functional and structural integrity of epithelial cells throughout the body is dependent upon an adequate supply of vitamin A. If this vitamin is deficient, differentiation switches in the pathway leading to keratinization of squamous metaplasia (Fig. 1.1). During the process of squamous metaplasia, mucous membranes change from a single layer of mucin-secreting and ciliated epithelium to multiple layers of epithelial cells, with overlying keratin resembling those of the skin (Moore, 1967; Toyoshima & Leighton, 1975).

The mode of action of vitamin A in normal epithelial differentiation and maintenance of the mucous membrane is not well defined. It is believed that retinoic acid is the major biologically active form of vitamin A in somatic epithelial cells, where the vitamin influences cellular differentiation (De Luca et al., 1971), while retinol is the stabilizer of biological membrances (Roels et al., 1969).

Epithelial tissues that depend upon retinoids for normal cellular differentiation and growth account for over half of the total primary cancer in both men and women. Epithelial cancer includes squamous metaplasia and carcinoma of a wide variety of organs and tissue sites in the body (Table 1.1).

1.1.3 Vitamin A Metabolism

Dietary vitamin A exists either as retinyl esters or as the provitamin β-carotene. Esters of retinol are hydrolyzed

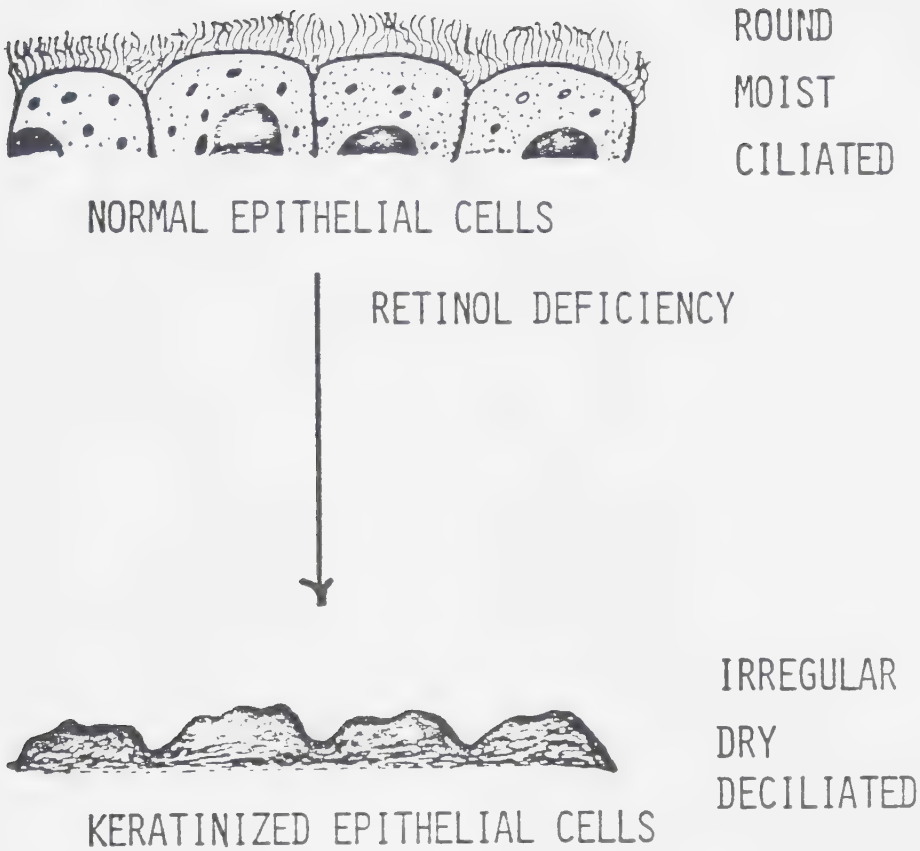


Fig. 1.1 EFFECT OF RETINOL DEFICIENCY ON THE MORPHOLOGY OF THE EPITHELIAL CELLS

TABLE 1.1 ORGANS AND TISSUE SITES WHICH ARE MOST SUSCEPTIBLE TO EPITHELIAL CANCER.

CONTACT EPITHELIA[1]	EXCRETORY EPITHELIA[2]	SECRETORY EPITHELIA[3]
SKIN(includes Melanoma)	COLON	PANCREAS
BUCCAL CAVITY & PHARYNX	RECTUM	BREAST
ESOPHAGUS	GALL BLADDER	PROSTATE
STOMACH	KIDNEY	TESTIS
SMALL INTESTINE	BLADDER	UTERUS
LARYNX		OVARY
TRACHEA,BRONCHUS & LUNG		THYROID
CERVIX UTERI(invasive)		

[1]Epithelia of tissues and organs which come into contact with external substances such as foods and chemicals.
[2]Epithelia of organs whose principal function is excretory.
[3]Epithelia of glands and organs regulated by hormones and immune system.

by specific esterases before retinol can be absorbed by intestinal mucosal cells (Ganguly, 1969). In contrast, β-carotene is first absorbed and then enzymatically cleaved to give two retinol molecules which are in turn reduced to retinol (Goodman & Huang, 1965). Before retinol can leave the intestinal mucosa, it must be esterified with long chain fatty acids preferentially palmitic acid. The resulting retinyl esters then pass into the lymphatics and are transported as chylomicrons to the blood from where they are taken up and stored by the liver (Huang & Goodman, 1965; Goodman et al., 1966). Eighty to ninety percent of the stored vitamin A in the human body is located in the liver, but substantial amounts are also found in the kidney and fat tissues and lesser amount in the plasma, small intestine, lungs and adrenals. In all tissues, retinyl esters are the major storage form. In contrast to tissue stores, over 90% of the plasma vitamin A occurs as retinol (Underwood, 1974; Mahadeven et al., 1965) (Fig. 1.2).

The mobilization and transportation of vitamin A from liver storage requires hydrolysis of the retinyl esters followed by conjugation of the free retinol with a specific, 20,000 molecular weight transport protein – retinol-binding protein (RBP) produced by the liver. The holoprotein is then released to the circulation where it binds to prealbumin as a 1:1 molar complex. The resulting complex transports retinol to target organs (Goodman, 1974; Peterson et al., 1974).

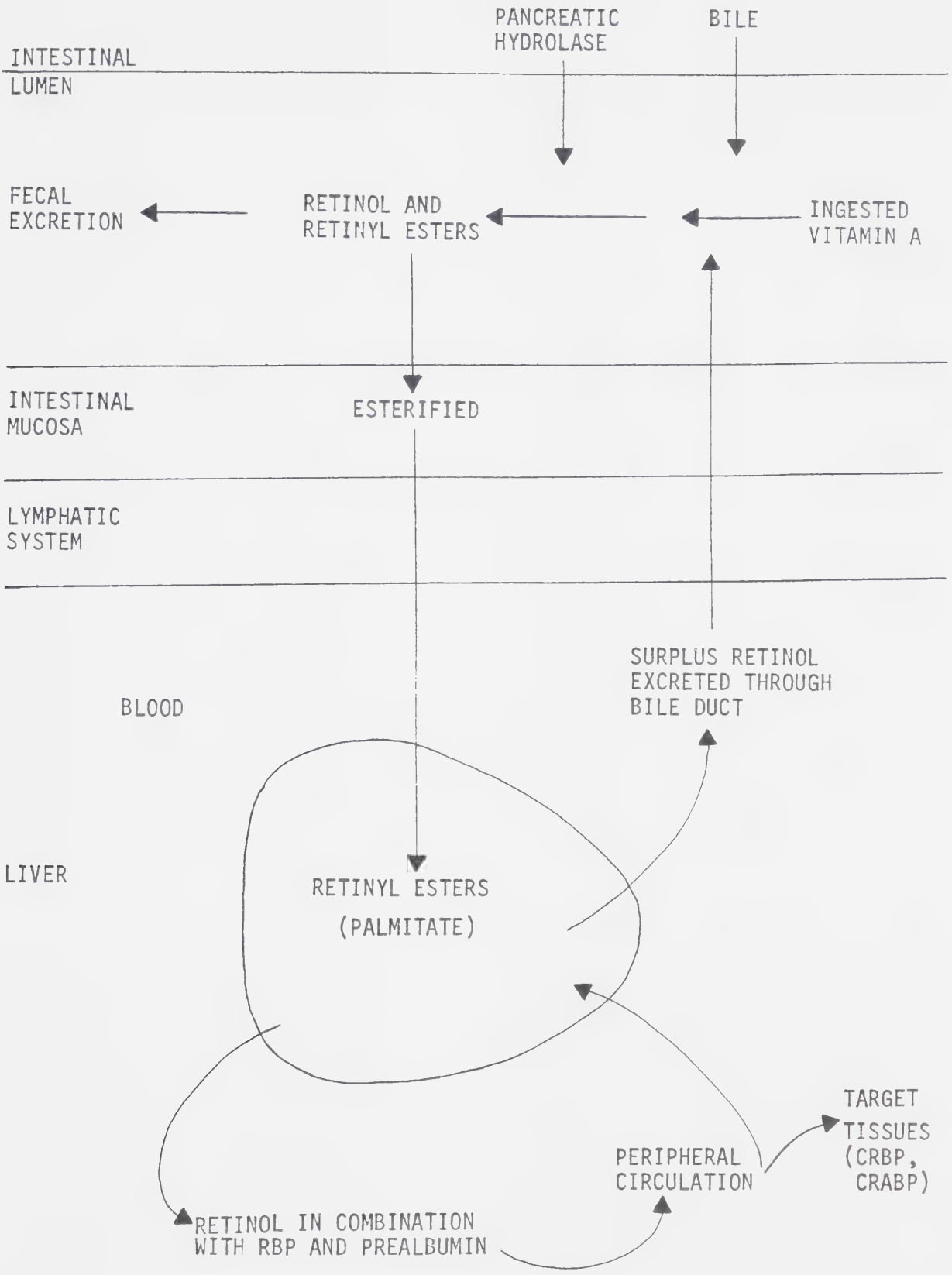


Fig. 1.2 VITAMIN A UPTAKE, STORAGE AND CIRCULATION

Rask and co-workers (1976) using isolated intestinal mucosal cells showed that the cellular uptake of retinol is mediated by a receptor. The mucosal cells readily accumulate labelled retinol from its complex with RBP without concomitant cellular uptake of the protein itself. The membrane receptor seems to recognize the protein rather than the retinol. During the uptake of the retinol, an altered form of RBP is generated, which cannot bind retinol and consequently prealbumin. It differs from holo-RBP, in that it lacks the terminal arginine residue. Heller and Chen (1977) using isolated pigment epithelial cells from bovine retina and labelled RBP also showed that the binding was at the cell surface without penetration of RBP into the cell. Therefore RBP is not only important for the transport of retinol in the blood, but is also an indispensable entity for recognition by the target cells and consequently for penetration of retinol through the plasma membrane.

1.1.4 Selected Characteristics Of Vitamin A In Man

Serum vitamin A is not in simple equilibrium with body stores and there is little relationship between serum and liver levels of vitamin A (Underwood, 1974; Meyer et al., 1942). Plasma levels of vitamin A thus do not provide a reliable index for predicting the vitamin A tissue levels. When dietary supplies are inadequate, liver stores are utilized to maintain a relatively constant blood level until stores are nearly exhausted, when this occurs, plasma levels

drop rapidly. In the absence of febrile or liver disease or protein malnutrition, it is possible that very low blood levels reflect depleted tissue reserves. However, acute infections and febrile disorders are associated with a transient reduction in plasma levels of vitamin A but liver stores are not necessarily depleted. Stress, in the form of infection or emotional factors, may influence plasma levels by decreased mobilization of hepatic stores, increased cellular need and increased renal loss (Underwood, 1974). Gastrointestinal disturbances which hinder the absorption of fats may be associated with decreased levels of vitamin A (Mahadeven et al.,1965). In contrast, elevated plasma and liver levels of vitamin A occur in the nephrotic syndrome and may reflect a decreased urinary loss and decreased catabolism by the kidney (Underwood, 1974).

Systemic data relating blood levels of vitamin A with levels of intake and with evidence of tissue deficiency in adults is limited.

During prolonged controlled depletion experiments with vitamin A carried out in human beings (Brenner & Roberts, 1943; Wald et al., 1969), the serum carotene levels decreased fairly rapidly in the early part of vitamin A depletion, while the serum vitamin A levels were resistant to change. It was noted that serum vitamin A levels are highly characteristic of the individual and less characteristic of vitamin A intake, whereas the plasma carotenoid levels differed little among individuals on

similar intake. In the classic Sheffield Study in World War II there was no significant difference between the plasma vitamin A levels of the deficient and the normal control groups even after the deficient group had been on approximately zero vitamin A intake for over one year (Hume & Krebs, 1949). In the few cases in which decrease in plasma vitamin A was actually found, it occurred only after signs of an acute deficiency had happened. In summary, it appears that the finding of low serum carotene levels reflect low intake, but that serum vitamin A levels may characterize individuals rather than reflecting intake.

Age and sex differences have been described. Males appear to have higher serum vitamin A levels than females and young children have lower serum levels than do adults. Serum carotene levels are reported to be greater in adult females than males (Pearson, 1967). Evidence in this regard is apparently sparse and not systemic.

1.1.5 Retinol And Carcinoma: Animal Studies

A connection between vitamin A and cancer was detected as early as 1926, (Fujimaki) when the development of carcinomas was found in rats fed on a vitamin A deficient diet. However, only recently have there been intensive efforts to investigate the possible role of vitamin A in relation to epithelial cancer.

Experimental studies have revealed that vitamin A deficiency is related to cancer of the stomach, nasopharynx,

lower respiratory tract and endocervix which are lined by glandular epithelium (Saffiotti et al., 1967; Chu & Malmgren, 1965). Vitamin A deficiency may change the glandular epithelium to squamous and whenever such "squamous metaplasia" occurs, there are grounds for suspecting increased risk of cancer development.

Convincing evidence of the significance of vitamin A in protecting against cancer has come from experimental studies in which tissues in organ culture or in intact animals are exposed to carcinogenic polycyclic aromatic hydrocarbons (PAH) to develop squamous cancer. Furthermore, a number of studies have shown that systemic prophylactic administration of vitamin A in its ester, alcohol or acid form, both before and after exposure to various chemical carcinogens (3-methylcholanthrene, 3-MC; benzopyrene, BP; dimethylbenz[a]anthracene, DMBA), inhibits the induction of metaplasia and carcinomas in various sites. Thus vitamin A protected rats against the early development of squamous neoplasms in response to 3-MC given by endo-tracheal instillation (Cone & Nettlesheim, 1973). Retinoid administration reduced the development of squamous metaplasia and carcinomas in rodents subjected to intra-tracheal administration of carcinogenic polycyclic hydrocarbons (Saffiotti et al., 1967). Supplemental vitamin A has been reported to prevent cancer of the forestomach and cervix in hamsters treated with polynuclear aromatic hydrocarbons (Chu & Malmgren, 1965). Vitamin A also

inhibits squamous metaplasia induced by BP in organ cultures of hamster tracheas (Crocker & Sanders, 1970).

The prevention of epithelial cancer by retinoids was demonstrated by Bollag (1975) using the classical two-stage skin carcinogenesis system. In this study, topical applications of the promoter, croton oil, induced benign papillomas which progressed to carcinomas. Oral retinoic acid, administered during the promotion phase, delayed the appearance, retarded the growth, and led to regression of papillomas. The appearance of carcinomas was also delayed and the incidence reduced. More recently, several experimental studies have included the inhibition of lung tumors in hamsters given intratracheal benzopyrene (Port et al., 1975) by oral 13-cis-retinoic acid (a synthetic retinoid analog) and the protection of rats against DMBA-induced breast cancer by oral retinyl methyl ether (Grubbs et al., 1977).

There is also evidence that carcinogens are more potent in vitamin A deficient animals. Various carcinogens bind more tightly to DNA in cultured tracheas from hamsters fed on a vitamin A deficient diet than from healthy animals (Genta et al., 1974). In the absence of vitamin A intake, the susceptibility of rats to pulmonary carcinogens was increased even in the presence of substantial liver stores of vitamin A and in the absence of deficiency symptoms (Nettlesheim & Williams, 1976).

Squamous metaplasia appears to occur as an early phenotypic change following exposure to a carcinogen. The similarity in appearance of the histological change of retinol deficiency and a carcinogen-induced squamous metaplasia leads to speculation that the two conditions are similar in more fundamental ways, namely, a) that a retinol-deficient epithelium is more prone to malignant change either "spontaneously" (i.e. without application of a specific carcinogenic agent) or in response to a carcinogen, and b) that retinol may reverse the early metaplasic changes induced by carcinogens and so inhibit or delay the appearance of carcinoma.

Indeed, animal studies have demonstrated that retinol deficiency increases susceptibility to chemical carcinogenesis in the respiratory system (Nettlesheim et al., 1975), skin (Davies, 1967), bladder (Cohen et al., 1976), and colon (Newberne & Rogers, 1973a). Small amounts of retinyl acetate or palmitate in the diet appear to abolish this enhanced susceptibility. Large doses of the natural retinoids have been reported to provide additional protection against carcinoma of the trachea and bronchus (Saffiotti et al., 1967), esophagus, stomach and intestine (Chu & Malmgren, 1965), lung (Cone & Nettlesheim, 1973) and breast (Moon et al., 1977).

1.1.6 Vitamin A And Cancer In Man

Although evidence based on laboratory animal models has become increasingly voluminous, information relating to humans is sparse. Nonetheless, the evidence to support a link between vitamin A deficiency and cancer in man comes from two sources – epidemiological studies of dietary intake and cancer incidence and biochemical studies involving comparison of serum vitamin A levels in cancer cases and controls.

1.1.6.1 Diet And Cancer Incidence

A number of studies have associated a relative deficiency of vitamin A intake with an increased risk of lung cancer. In 1975, Bjelke reported results of a five-year follow-up study involving 8,278 men who had responded to a questionnaire on their smoking and dietary habits. An index of vitamin A intake, essentially based on reported consumption of carrots, eggs and milk, was negatively associated with lung cancer incidence at all levels of cigarette smoking. The relative risks (RR) of lung cancer for a low vitamin A index were greatest in heavy smokers (RR=2.86), less for light smokers (RR=2.27) and close to unity for non-smokers. The overall RR was 2.63 ($p<0.01$). Higher risks were found when incidences of histologically confirmed carcinomas – other than adenocarcinomas – were compared.

More recently, Hirayama (1979) reported results of a 10-year follow-up study involving 2,417,844 Japanese who had responded to questionnaires as to their diet and smoking habits. Eight hundred and seven deaths from lung cancer were recorded, the RR was found to be reduced by half in those who consumed green-yellow vegetables daily as compared to non-consumers. The consumers of green-yellow vegetables showed lower risk of lung cancer in both smokers and non-smokers, and this was true for both males and females. A similar report was made by MacLennan and his colleagues (1977) who found in a case-control study that low consumption of green vegetables was related to lung cancer in both sexes. The estimated risk of low versus high vegetable intake was 2.23.

A research group, at the Roswell Park Memorial Institute in the United States, conducted a number of studies concerning vitamin A intake and cancer. These studies indicated that individuals with a lower vitamin A intake had a higher incidence of cancer of the bladder (Mettlin & Graham, 1979), lung (Mettlin et al., 1979), and larynx (Graham et al., 1981). One of the case-control studies they have conducted was the association between vitamin A intake and the RR of lung cancer (Mettlin et al., 1979). Retrospective dietary and smoking data were collected from interviewing 292 patients with lung cancer and 801 control patients, who

had neither cancer nor disease of the respiratory system, during 1957-1965. Vitamin A intake was estimated from interviews with patients as to their usual frequency of consumption of 21 different food items which were considered to be rich in vitamin A during the 12 months prior to the onset of symptoms. The vitamin A content of the diet was calculated using the U.S. Department of Agriculture tables of food values (Watt & Merrill, 1968). The Mantel-Haenszel (1959) age- and smoking-adjusted RR for these patients with a lower vitamin A intake was 1.7 times greater than the controls ($p<0.05$). Increased risk of bladder cancer together with a low index of vitamin A intake has been reported by Mettlin and Graham (1979). Over five hundred bladder cancer patients were compared with at least one thousand age-matched controls, and a RR of 2.07 ($p<0.01$) was obtained for those with a low versus high vitamin A index. Milk and carrot intakes were the major sources of ascertained vitamin A intake.

These studies present a consistent picture as to the relationship between low vitamin A intake and increased RR of cancers of epithelial cell origin. In most of the studies, specific interest in vitamin A arose only after completion of an investigation undertaken for reasons unrelated to vitamin A status, so that only some of the food sources of vitamin A were investigated, such as milk, carrots and green-yellow

vegetables. The omissions of foods, such as liver, fortified margarine and any vitamin supplements used may have resulted in an underestimation of the RR for vitamin A. Therefore, it is of paramount importance that the evidence of the association between vitamin A intake and cancer be substantiated by biochemical assessment of vitamin A status.

1.1.6.2 Plasma Vitamin A Levels And Cancer - Clinical Studies

Subnormal plasma vitamin A has been observed in cancer of some epithelial tissues such as gastrointestinal (GI) tract (Abels et al., 1941), bronchus (Basu et al., 1976), oropharynx (Ibrahim et al., 1977) and lung (Atukorala et al., 1979). Comparisons of the plasma retinol levels between control patients and newly diagnosed cancer patients in both developed and developing countries, such as Britain (Basu et al., 1976; Atukorala et al., 1979), United States (Abels et al., 1941; Cohen et al., 1977), India (Wahi, 1962), Pakistan (Ibrahim et al., 1977) and East Africa (Clifford, 1972), have revealed lower retinol levels in cancer patients than in controls.

In a study of 17 cases of nasopharyngeal carcinoma in East Africa (Clifford, 1972), levels of both plasma vitamin A and carotene were significantly lower in those with cancer than controls ($p<0.05$). In the study of oral and oropharyngeal cancers in Pakistan by Ibrahim et

al. (1977), the plasma retinol levels were highly and significantly lower (by 50%) in 203 cancer patients than in 112 controls. Wahi et al. (1962) found the same trend in India. A case-control study by Basu et al. (1974) involving 35 hospitalized terminal cancer patients and 10 non-cancerous hospitalized controls showed a linear relationship between serum cholesterol and vitamin A. Both parameters were low in the cancer patients. A study involving 28 newly diagnosed cases of bronchial carcinoma, 10 healthy controls and 9 patients with non-malignant bronchial disease showed that the bronchial carcinoma patients had vitamin A levels substantially and significantly lower than in the controls ($p<0.01$) (Basu et al., 1976). In 1941, Abels et al. reported a case-control study of hospitalized cancer patients including 51 GI (28 gastric, 18 rectal, and 5 esophageal cancer), 48 leukemia, 21 Hodgkin's disease, 9 pancreas, and 6 bone sarcoma patients. All of these patients had mean plasma vitamin A levels appreciably lower than a selected sample of healthy controls.

In recent years, there have been reports suggesting that lower vitamin A may predispose an individual to a higher risk of cancer (Kark et al., 1981; Wald et al., 1980; Haines et al., 1982). Kark and Wald analyzed the total retinol levels in stored blood samples, taken for unrelated research purposes from people living in the

U.S. and U.K., who were apparently free of cancer at that time. In both of these studies, people with total blood retinol levels near the top of the normal range were at significantly lower risk of developing cancer over the subsequent few years than those with levels near the bottom of the normal range. Kark et al. followed a total of 3,102 individuals (both blacks and whites) in Evans County in Georgia for 12 - 14 years. Blood samples were taken upon entry to the study, and were stored at -20°. One hundred and twenty-nine new cases of cancer developed over the 14 years, and 85 persons survived until the end of the study. The results from this study indicated that those with low plasma retinol values had a six-fold increased risk of developing cancer in subsequent years. This association was independent of age, smoking habits and plasma cholesterol.

The retrospective study conducted by Wald et al. involved 16,000 males, aged 35 - 64 years. These people attended a research centre for a comprehensive health-screening examination between March, 1975 to December, 1978. Blood samples were taken and the sera were stored at -40°C. By the end of 1976, 86 men had developed cancer. Controls were 172 males chosen from the remainder study population who were alive and without cancer. Mean retinol for all cancer patients was significantly lower ($p<0.025$) than for controls with

the difference being greatest for GI and lung cancer. RR of cancer associated with retinol levels at the lowest quintile was 2.2 times that in the highest quintile. These authors suggested that plasma retinol levels may have a predictive value for subsequent cancer.

1.2 Conclusion And Plan Of Present Study

Both retrospective and prospective data have consistently provided evidence that vitamin A deficiency may be associated with metaplastic changes in epithelial tissues, especially in GI, respiratory and urogenital tracts, and that these changes may later progress to neoplasia. Studies on experimental animals have indicated that such changes may even be reversed by natural and synthetic retinoids.

The association between vitamin A and epithelial cancer in man is also evident from the results of epidemiological studies. Studies of both dietary intake and serum levels have shown an inverse relationship between vitamin A and the incidence of cancer, thus, implicating the significance of vitamin A status in the prediction of cancer risk. A majority of the studies reported in the literature have been concerned with cancer of the lung. Reports on human subjects with colorectal cancer alone are scanty. However, experimental studies with animals have revealed an increased incidence of dimethylhydrazine (DMH)-induced colon carcinoma

in rats deficient in vitamin A (Newberne et al., 1973b), and that a marginal dietary level of vitamin A not only enhanced liver cancer in rats exposed to aflatoxin B_1 but resulted in a 29% incidence of colon cancer as well (Newberne & Rogers, 1973a). In view of the fact that colorectal cancer is a disease which has a high incidence in the humans, as well as a poor survival rate, it is of paramount importance that the relationship between vitamin A and colorectal cancer be studied.

The present study was undertaken to investigate the plasma levels of vitamin A and the factors involved in its metabolism in patients with colorectal cancer who appeared to be disease-free after surgery.

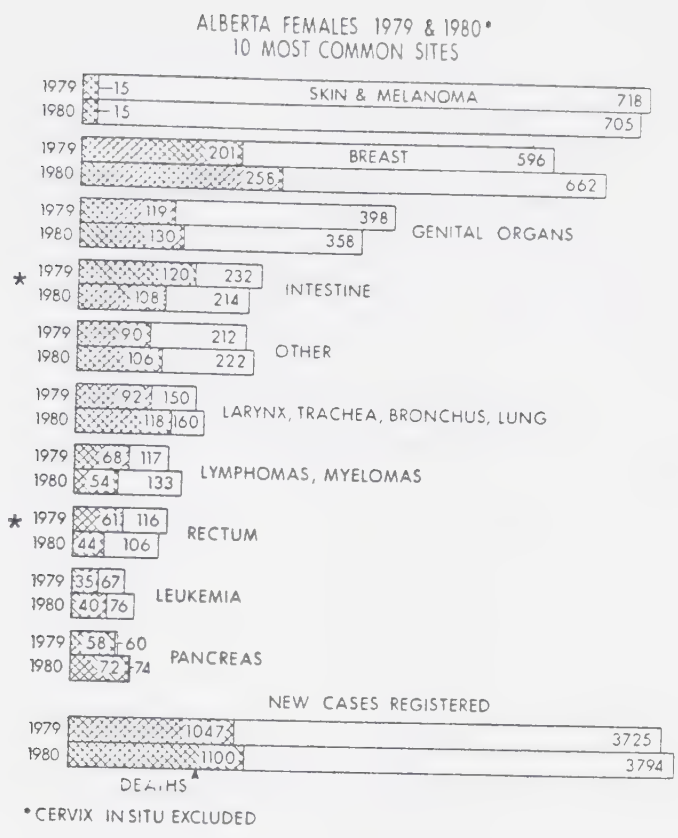
Chapter 2

METHODOLOGY

2.1 INTRODUCTION

In Canada, the incidence of gastrointestinal (GI) malignancy is second only to skin cancer, and the mortality rate is second only to cancer of the lung. Overall it is the second most fatal cancer for men and women, and it has been estimated that, each year, about 15,400 Canadians will be afflicted with this malignancy (Lim,1979). By far the most common GI malignancy is colorectal carcinoma which accounts for over 52% of the new cases. Only 41% of all detected colorectal cancers are in a localized stage, i.e. without nodal involvement. The five-year survival for localized colorectal carcinoma is over 70% versus 40% for advanced lesions. (ACS, 1975).

The average incidence rate for colorectal cancer for 1976-1980 in Alberta was 31.2 and 30.5/100,000 for men and women, respectively. The average mortality rate was 15.8 and 16.1 for men and women, respectively (PCHB, 1981a). In 1980 alone, 256 new cases and 131 deaths for rectal cancer and 417 new cases and 202 deaths for colon cancer were reported out of a population of 2.14 million (Fig. 2.1) (PCHB, 1981b). Thus about 50% of those developing colorectal cancer will die of it. Most of these deaths occurred in patients with bowel plus regional node involvement and/or metastastic spread (Carter, 1976).



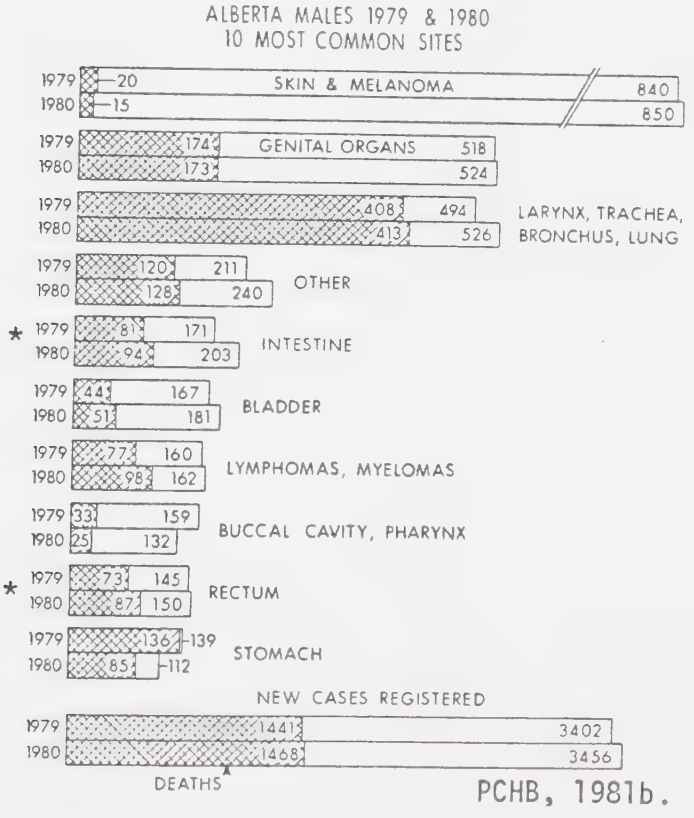


Fig. 2.1 NO. OF NEW CASES AND DEATHS OF COLORECTAL CANCER*, 1979-1980. (With permission from Provincial Cancer Hospitals Board.)

A 10-year follow-up of 487 cases of colorectal cancer at the W. W. Cross Cancer Institute (WWCCI) in Edmonton, Alberta showed an over-all five-year survival of 35% (McCarten, 1973). Accordingly, with this dismal survival figure, an ongoing randomized, prospective surgical adjuvant GI trial involving Dukes' B2 and C colorectal carcinoma and gastric carcinoma was initiated in 1976 at the WWCCI. Patients entering into this trial were randomly assigned into one of the three groups, namely :

1. control, i.e. receiving no adjuvant treatment,
2. immunotherapy (BCG),
3. chemo-immunotherapy (MeCCNU, 5-Fu, BCG).

All patients considered for the study were entered as randomized only if informed consent was given. Patients were ineligible if they had had pre-operative radiotherapy, chemotherapy or immunotherapy within the previous year. The main assessment of the trial is survival which will be calculated for each patient allocated from the date of definite resection.

The present study was an integral part of the WWCCI trial. The main objective of the study was to focus on the relationship between vitamin A and the recurrence of colorectal cancer. The hypothesis to be tested was whether lower plasma vitamin A is associated with increased risk of cancer recurrence. In addition to vitamin A per se, RBP, cholesterol, cortisol and proteins were measured. The rationale for the inclusion of these parameters is outlined

below : –

The plasma levels of vitamin A may be affected by the concentration of the circulating carrier protein. Vitamin A is transported in the blood associated with a specific protein, RBP which is in turn bound to prealbumin and circulates as a 1:1 molar complex. It is possible, therefore, that low circulating levels of the vitamin could be due to a decreased availability of the carrier protein.

The absorption of vitamin A and its precursors may be affected by impaired fat absorption from the gut. Since cholesterol is also a fat soluble substance, the measurement of this parameter may provide some hints as to the efficiency of absorption of this vitamin. Moreover, current theories regarding cancer causation have generated interest in plasma cholesterol levels as potential causal factors in the genesis of colon cancer. Recently, the relationship between cholesterol and cancer has become both acute and controversial. Some investigators reported a significant link between low cholesterol levels and subsequent cancer (Rose et al., 1974; Williams et al., 1981; Peterson et al., 1981; Kagan et al, 1981; Garcia-Palmieri et al., 1981) while others have not found such an association (Dyer et al., 1981; Kozarevic et al., 1981; Thomas et al., 1982). Most of these reports have come from studies originally designed to identify factors which precede the appearance of coronary heart disease. Therefore, the measurement of plasma cholesterol in this study may add more information to this

controversial issue.

Stress is known to increase the vitamin A requirement by increasing its degradation mediated by the secretion of adrenocorticotrophic hormone (ACTH) which is antagonistic to vitamin A. The presence of tumor cells could be considered as a state of stress, which may lead to the outpouring of ACTH, this may in turn cause a loss of vitamin A by favouring its elimination from the body.

Various studies have indicated that depressed plasma vitamin A levels may be associated with protein deficiency. Several workers have demonstrated a decrease in plasma vitamin A levels in children with protein-calorie malnutrition (Smith et al., 1973a; Ingenbleek et al., 1975). Moreover, supplying calories and protein without supplemental vitamin A resulted in a clinical cure of the vitamin A deficiency symptoms and a significant rise in vitamin A in the blood (Smith et al., 1973a). Malnutrition is often seen in patients with advanced malignant disease (Theologides, 1977), therefore, it is possible that the decreased vitamin A levels may be a manifestation of generalized nutritional deficiency and not merely a deficiency of vitamin A.

A classical cohort study in which plasma vitamin A levels were examined in the total population and followed them in a prospective fashion for the development of cancer would be desirable. However, such a design would be expensive and lengthy. Therefore, the present study

addressed the question more economically by using patients already being followed as part of a clinical trial.

2.2 STUDY POPULATION

2.2.1 Patients

A total number of 103 patients who were on the control arm of the WWCCI surgical adjuvant GI trial was assigned to this study. They were admitted to the WWCCI between February, 1976 and January, 1982. All these patients had previously undergone curative resections of histologically proven colonic and rectal adenocarcinomas. Sixty-six patients had Dukes' B2 tumor and 37 had Dukes'C tumor. Surgical-pathologic staging, based on the depth of penetration of carcinoma of the colon or rectum, conformed to a modification of Dukes' Classification (1932) : a Dukes' B2 tumor involves the full thickness of the bowel wall but no nodal involvement, and a Dukes' C tumor has regional lymph-node metastases. At the time of diagnosis and surgery, the mean age of the entire group was 62.6 ± 1.3 years, with a range between 23 and 80 years. Ninety-eight and one-tenth percent of the patients were over 40 and 1.9 percent below 40 years. Fifty-four of them were men and 49 were females, with a male : female ratio of 1.1 : 1 (Table 2.1).

TABLE 2.1 AGE AND SEX DISTRIBUTION OF THE STUDY POPULATION.

GROUPS	AGE RANGE (Years)	NO OF MALES	NO OF FEMALES	M/F RATIO*
CONTROLS (n=65)	23 - 65 (46 5 ± 1.4)	34 (47.8 ± 1.9)	31 (45.1 ± 2.0)	1.1 : 1
COLORECTAL B2 (n=66)	23 - 80 (62.4 ± 1 2)	36 (62.3 ± 1.3)	30 (62.7 ± 1.8)	1.2 : 1
COLORECTAL C (n=37)	39 - 80 (62.9 ± 1.6)	18 (65.4 ± 2.5)	19 (60.6 + 2.0)	0.9 : 1
COLORECTAL B2 + C (n=103)	23 - 80 (62.6 ± 1.3)	54 (63.3 ± 1.2)	49 (61.9 ± 1 5)	1.1 : 1

Mean age ± SEM in brackets.
*Male/Female ratio

2.2.2 Controls

Sixty-five apparently healthy subjects who were Red Cross blood donors from various locations in Northern Alberta (both urban and rural) formed the basis of the controls of this study. Their mean age was 46.5 ± 1.4 years, with a range between 23 and 65 years. Seventy-two and three-tenths percent of the controls were over 40 years and 27.7 percent below 40. Thirty-four were men and 31 were females, with a male : female ratio of 1.1 : 1 (Table 2.1).

One may argue that a better set of controls would be patients without malignant disease who had undergone large bowel surgery. However, the surgical operation itself may leave the patients with a reduced capacity to absorb nutrients. This would make the evaluation of the results difficult, therefore, healthy subjects were used as controls. Although such subjects were not an ideal "control" group, their biochemical values provided normal values for comparison with the results from the cancer patients since the same methods were employed in determining the various indices for both groups.

2.3 BIOCHEMICAL MEASUREMENTS

All biochemical measurements were performed blind, i.e. the classification of the patients and their clinical characteristics were not made known during this phase of the study.

2.3.1 Sample Collection

Non-fasting blood samples (10 ml heparinized blood from each patient or donor) which were drawn at 10.00 a.m. everyday, were collected from the WWCCI Clinic and the Red Cross Blood Transfusion Clinic from October, 1981 to February, 1982. The collection tubes were wrapped with foil during transportation from the clinics to the laboratory thus minimizing the loss of vitamin A , since this vitamin is extremely sensitive to and will be destroyed by light. The tubes were centrifuged at 2,000 rpm for 10 minutes in a Sorvall Superspeed RC 2-B Automatic Refrigerated Centrifuge. Plasma samples were separated and stored in tubes, wrapped with foil at -35°C until analysed. The analyses for vitamin A and other biochemical parameters were carried out six months to one year after the collection of the samples.

The effect of long-term storage on vitamin A constancy and estimation is definitely of great concern since repeated freezing and thawing may possibly affect plasma vitamin A levels. One study showed that after 4 1/2 months of storage at -20°C there was no significant change in vitamin A content (Bessey et al., 1949). More recently, the study of Kark et al. (1981), using the Evans County sera which had been stored for a period of 14 - 16 years, indicated that repeated thawing and refreezing had no recognizable affect on vitamin A content.

2.3.2 Determination Of Plasma Vitamin A

Vitamin A was determined by a modification of the fluorometric method of Hansen and Warwick (1969). They measured the fluorescence of vitamin A at an excitation wavelength of 340 nm and an emission wavelength of 480 nm. However, there was considerable interference from carotenoids at this wavelength (Thompson et al., 1973; Steveninck & de Goeij, 1973). Steveninck and de Goeij suggested the measurement of fluorescence at an emission wavelength of 550 nm, where interference from carotenoids is virtually zero. Therefore, the fluorescence in this study was measured accordingly at an emission wavelength of 550 nm. The intensity of fluorescence increased linearly with concentration (Fig. 2.2) and there was no interference from carotenoids.

All the preparation and measuring procedures were performed in dim light since vitamin A is sensitive to light at certain wavelengths (Bessey et al., 1946).

All-trans retinyl acetate (Sigma) in absolute ethanol was used as the standard for vitamin A (range : 0.5 - 2.0 mcg/ml). The acetate form was used because of its greater stability and comparable fluorescence with vitamin A alcohol (Hansen & Warwick, 1969).

Aliquots (0.2 ml) of plasma were pipetted into 15 ml Sovril tubes fitted with teflon lined screw caps. The same volumes of distilled water or standard were used instead of plasma in the blank and the standard, respectively. One

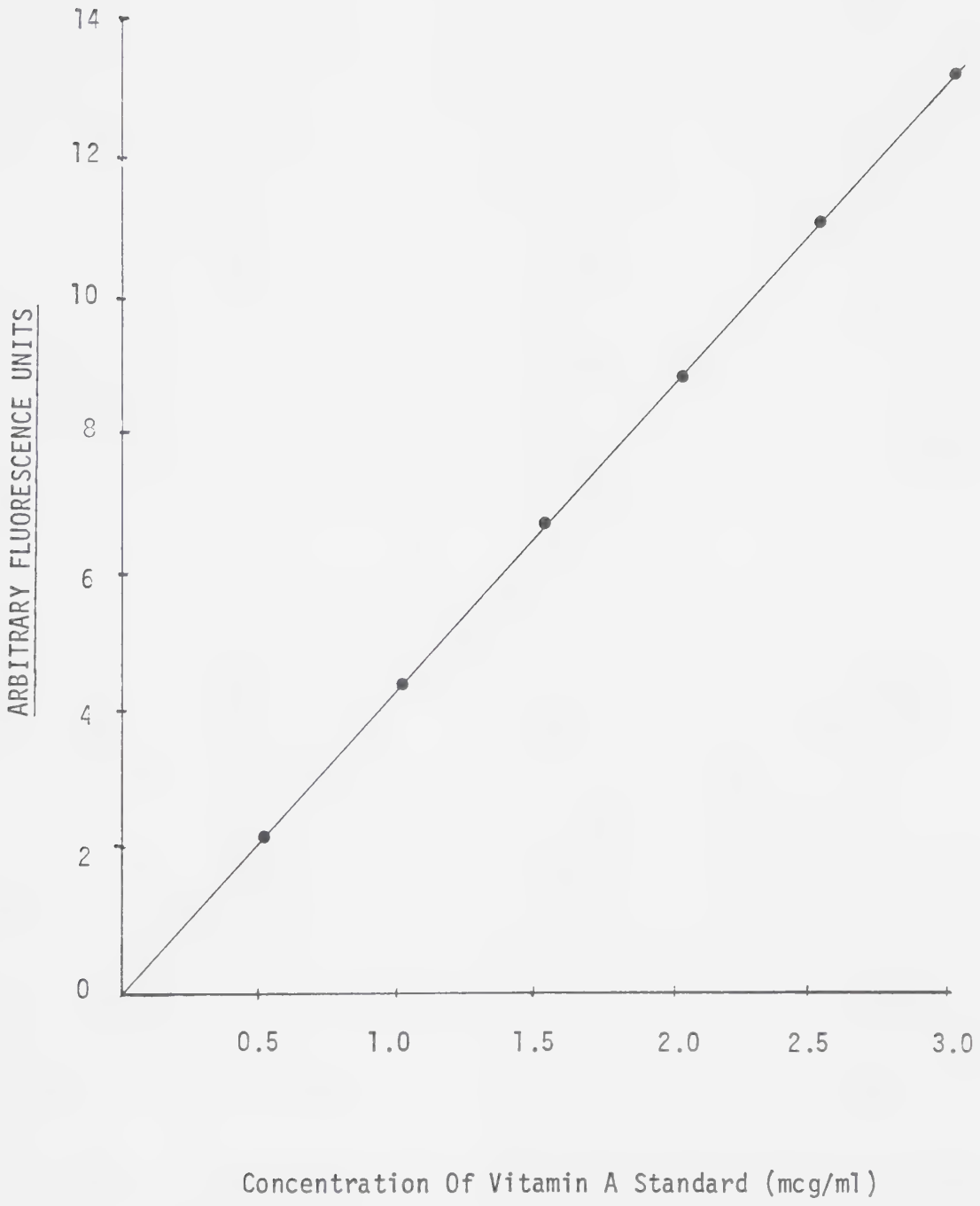


Fig. 2.2 STANDARD CURVE FOR VITAMIN A DETERMINATION

milliliter of distilled water was added to each tube and mixed and 2.0 ml of absolute ethanol was added slowly, with mixing, to precipitate proteins. Five milliliters of High Pressure Liquid Chromatography (HPLC) grade hexane was then added. The tubes were capped and mixed for 30 seconds on a vortex mixer to ensure complete extraction of vitamin A from the aqueous ethanolic phase to the hexane layer. The tubes were then centrifuged at 2,500 rpm for 5 minutes. The upper hexane layer was removed and transferred into a fluorometric cell by using a Pasteur pipette and its fluorescence was measured in a Perkin Elmer 650-10S Fluorescence Spectrophotometer.

All plasma samples were measured in triplicate and the average reading was used to calculate the plasma vitamin A (retinol) content using a conversion factor of 0.3/0.344 since retinyl acetate was used as the standard in the analyses. Plasma samples were divided into 27 batches, 8 samples in each of the first 23 batches and 9 samples in each of the last 4 batches. Two batches were run on each day, except for the last day when only 1 batch was run. A standard (1 mcg/ml) and a blank were included in each run.

2.3.3 Determination Of Plasma Cholesterol

The method of Watson (1960) was employed in the determination of plasma cholesterol. This procedure is a colorimetric assay in which the reagent 2,5-dimethylbenzene sulphonic acid replaces p-toluene sulphonic acid in the

method of Pearson et al. (1953). All reagents used were of analytical grade. Pure cholesterol (Kodak) dissolved in glacial acetic acid was used as the standard (range : 1 - 4 mg/ml). The intensity of the absorbance increased linearly with concentration (Fig. 2.3).

Aliquots of 0.1 ml of plasma were placed into tubes of approximately 2 cm diameter in order to facilitate mixing, 0.1 ml of distilled water and standard (4 mg/ml) were also set up for the blank and standard respectively. One-tenth of a milliliter of glacial acetic acid was added to the plasma and the blank, but 0.1 ml of distilled water was added to the standard instead. Two and a half milliliters of reagent mixture (3 volumes of acetic anhydride with 1 volume of the 2,5-dimethylbenzene sulphonic acid solution and 1 volume of glacial acetic acid) was added to each tube and mixed with a vortex mixer. Each tube was allowed to cool for 10 - 15 minutes. Three-tenths of a milliliter of sulphuric acid was then added to each tube and the contents were agitated immediately until all precipitates had completely dissolved. The tubes were allowed to stand in the dark for color development. The optical density of each sample was measured against the blank exactly 20 minutes after the addition of sulphuric acid using a UNICAM SP1800 Spectrophotometer.

The samples were divided into 22 batches, 10 samples in each batch and 2 batches were run on every single day, a blank and a standard were included for each run to check for

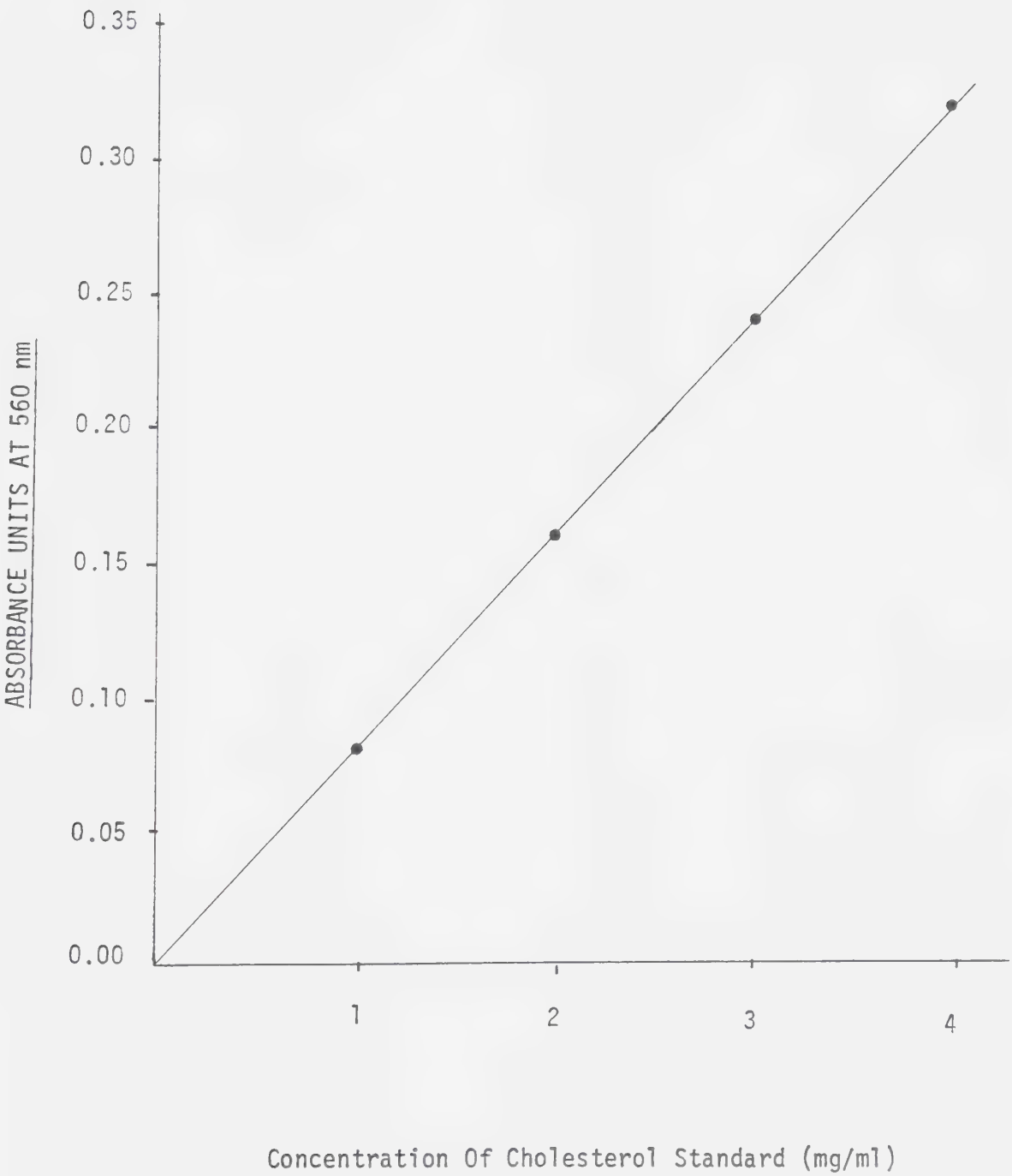


Fig. 2.3 STANDARD CURVE FOR CHOLESTEROL DETERMINATION

the daily variation and the stability of the machine. All samples were measured in duplicate and an average reading was used in the calculation of the amount of cholesterol in the plasma.

2.3.4 Plasma Cortisol Determination

Mattingly's method (1962) was employed for the determination of plasma cortisol. Free and protein-bound corticosteroids were extracted from the plasma with methylene chloride. The organic extract was shaken with a sulphuric acid-ethanol reagent. After removing the supernatant dichloromethane, the resulting fluorescence of the acid was compared with that of a known concentration of cortisol treated in the same manner. Maximum fluorescence of corticosteroids is produced by excitation at 475 nm and maximum emission of fluorescence occurs at 530 nm.

High pressure liquid chromatography (HPLC) grade methylene chloride was used instead of the BDH grade as suggested by Mattingly since the latter needs repurification before use and the steps are tedious and time consuming. Fluorescence reagent was prepared by adding 7 volumes of concentrated sulphuric acid to 3 volumes of absolute ethanol in a flask which was kept cold in iced water. Cortisol standard was prepared by dissolving 50 mg corticosterone (Sigma) in 50 ml absolute ethanol. One milliliter of this solution was diluted to 100 ml with distilled water (10 mcg/ml). These solutions were kept at 4°C and remained

stable for months. A range of standards (1 - 5 mcg/ml) was prepared from the 10 mcg/ml solution, the intensity of fluorescence increased linearly with concentration (Fig. 2.4).

All glassware was washed with chromic acid, followed by thorough rinsing with tap water and finally with distilled water.

To an aliquot (0.2 ml) of plasma in a 15 ml Sovril tube, 1 ml of distilled water and 4 ml of methylene chloride were added. A reagent blank and a standard containing distilled water and corticosterone respectively were carried through the procedure. All the tubes were stoppered and shaken very gently by hand for 10 minutes. The tubes were then centrifuged for 2 minutes at 2,000 rpm and the supernatant plasma was removed by suction. An aliquot (3 ml) of the methylene chloride extract was transferred to a stoppered tube. At zero time, 1.5 ml fluorescence reagent was added to the blank and mixed vigorously for 20 minutes on a vortex mixer. This procedure was repeated by adding the fluorescence reagent at one minute intervals to the standard and the extracts of plasma. The methylene chloride layer was sucked off from each tube in turn, starting with the blank. The fluorescence of each solution was measured at exactly 15 minutes after mixing with the fluorescence reagent in a Perkin-Elmer 650-10S Fluorescence Spectrophotometer.

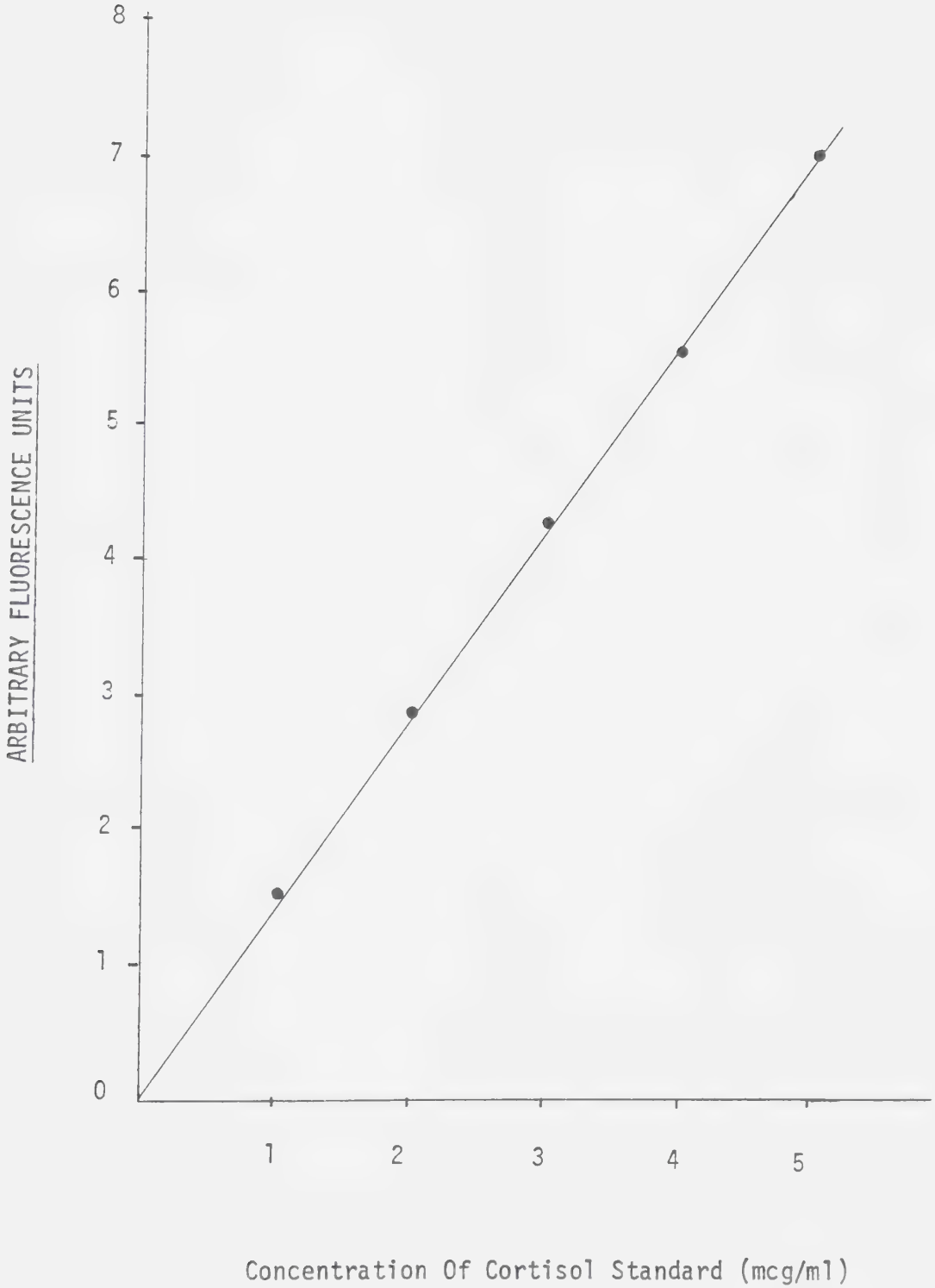


Fig. 2.4 STANDARD CURVE FOR CORTISOL DETERMINATION

The samples were divided into 55 batches. Fluorometry was performed in batches of not more than 4 samples (in duplicates), a blank and a standard (1 mcg/ml) since timing is very important to keep the non-specific fluorescence as low and as uniform as possible.

2.3.5 Determination Of Total Proteins In The Plasma

Total plasma proteins were determined by the method of Gornall et al. by means of the Biuret reaction. This reaction is based on the principle that the protein of the plasma will react with an alkaline solution of cupric ions and the intensity of the purple color produced is proportional to the amount of protein present in the plasma (Fig. 2.5).

Standard protein solution (Sigma) was used as the standard and Biuret reagent (containing 0.15 percent copper sulphate, 0.6 percent sodium potassium tartrate and 3 percent sodium hydroxide) was prepared as suggested by Gornall.

Aliquots of 0.1 ml of plasma were pipetted into 15 ml test tubes. The same volume of standard and 0.9 percent saline as plasma were used, in the standard or blank, respectively. Two milliliters of saline and 0.8 ml of Biuret reagent were added, to each tube and mixed with a vortex mixer. The tubes were allowed to stand at room temperature for 30 minutes. All samples were measured in duplicate. A blank and standard (6 g/dl) were included in

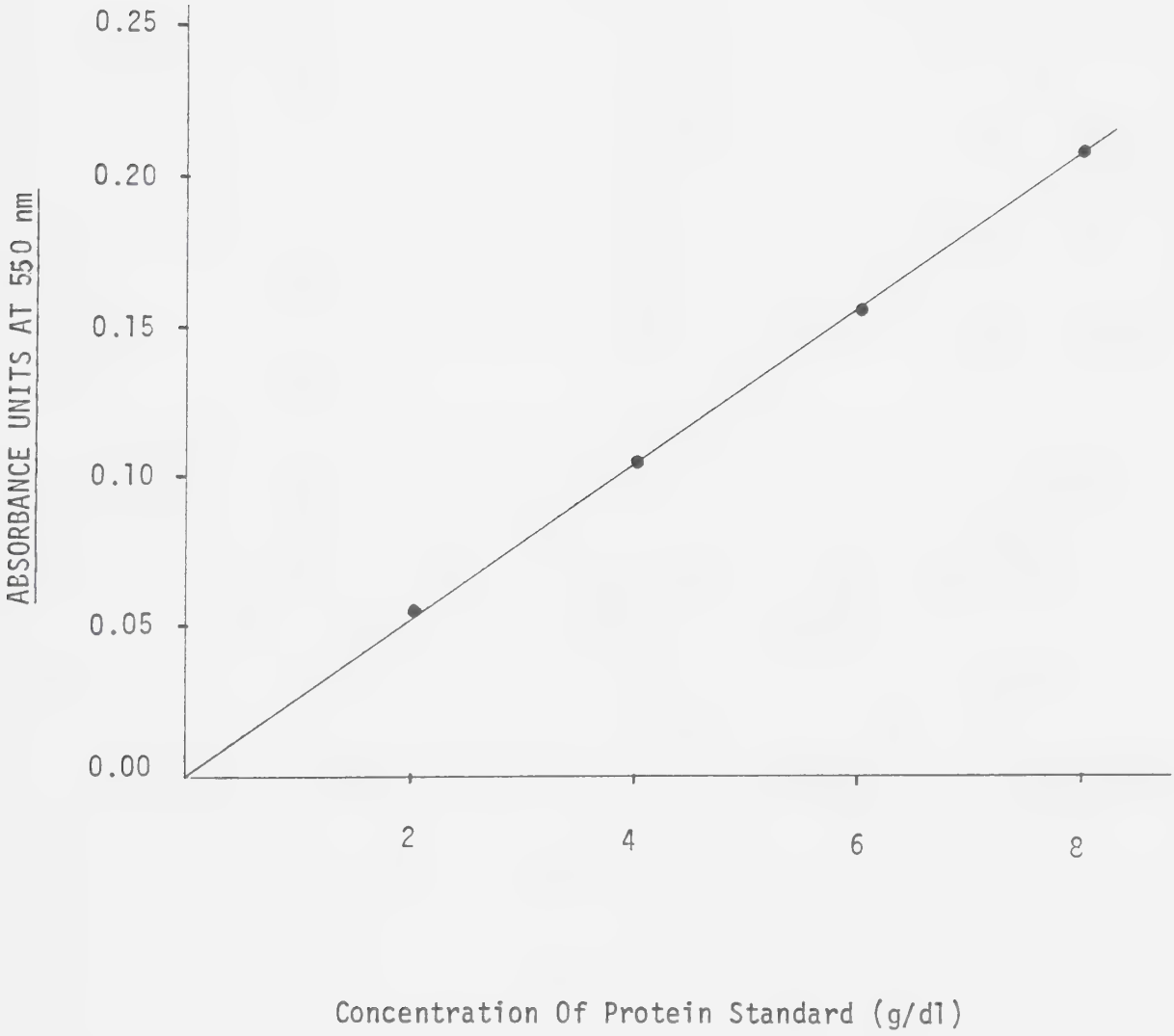


Fig. 2.5 STANDARD CURVE FOR THE DETERMINATION OF TOTAL PROTEINS

each run.

Absorbance was measured at 550 nm using the UNICAM SP1800 Spectrophotometer.

2.3.6 Plasma Albumin Determination

The method of Doumas et al. (1971) was employed for the determination of plasma albumin. This is a fast and reliable method using a dye-binding technique. The dye used was Bromcresol Green. The absorbance-concentration relationship is linear for samples containing up to 6 g/dl (Fig. 2.6). Bovine standard albumin, fraction V (Sigma) was used as the standard.

An aliquot of 25 mcl of plasma was added to a 15 ml test tube, 5 ml of working dye solution was then added and mixed on a vortex mixer. A reagent blank and a standard containing water and albumin standard (6 g/dl), respectively, were included in each run.

The absorbance was measured at 628 nm in a UNICAM SP1800 Spectrophotometer. The concentration of the plasma albumin was calculated from the average reading of the duplicate samples.

2.3.7 Plasma Globulin Determination

The globulin concentration in the plasma was obtained from the difference between total protein and albumin concentration.

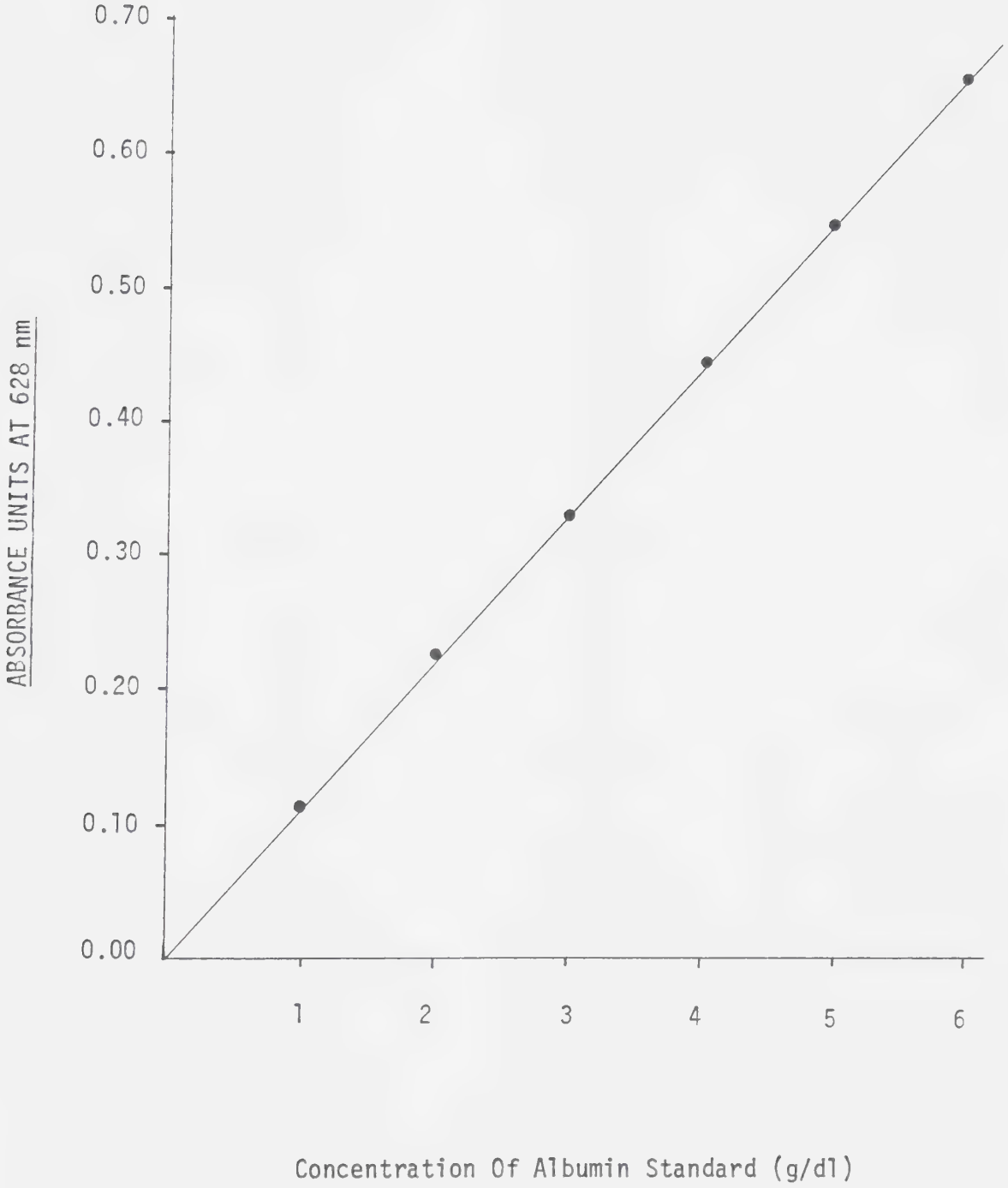


Fig. 2.6 STANDARD CURVE FOR ALBUMIN DETERMINATION

2.3.8 Determination Of Retinol-binding Proteins In the Plasma

The single radial immunodiffusion technique (Mancini et al., 1965) was used in the determination of retinol-binding protein. Standardized and stabilized human serum (Behring Diagnostics) with known protein concentration was used as the standard and for the construction of a standard curve from which the protein concentration of the plasma samples were obtained. (Fig. 2.7) Physiological saline (0.9%) was used for dilution of the standard serum solution and the samples.

LC-partigen immunodiffusion plates (Behring Diagnostics) were used for the assay. The plates contained 12 wells. Aliquots (20 ml) of standards (range 1:1, 1:1.5, 1:2, 1:4) were placed into the first 4 wells, whereas the same volume of diluted plasma (1:4) was placed into the remaining wells using an Eppendorf micropipette. The plates were allowed to stand for 10 - 20 minutes, then closed and incubated for 72 hours at room temperature.

The diameters of the precipitation rings were measured in a measuring viewer made by Behring Company. The concentration of retinol-binding protein in the test sample was obtained directly from the plot of the square of the diameter on the standard curve.

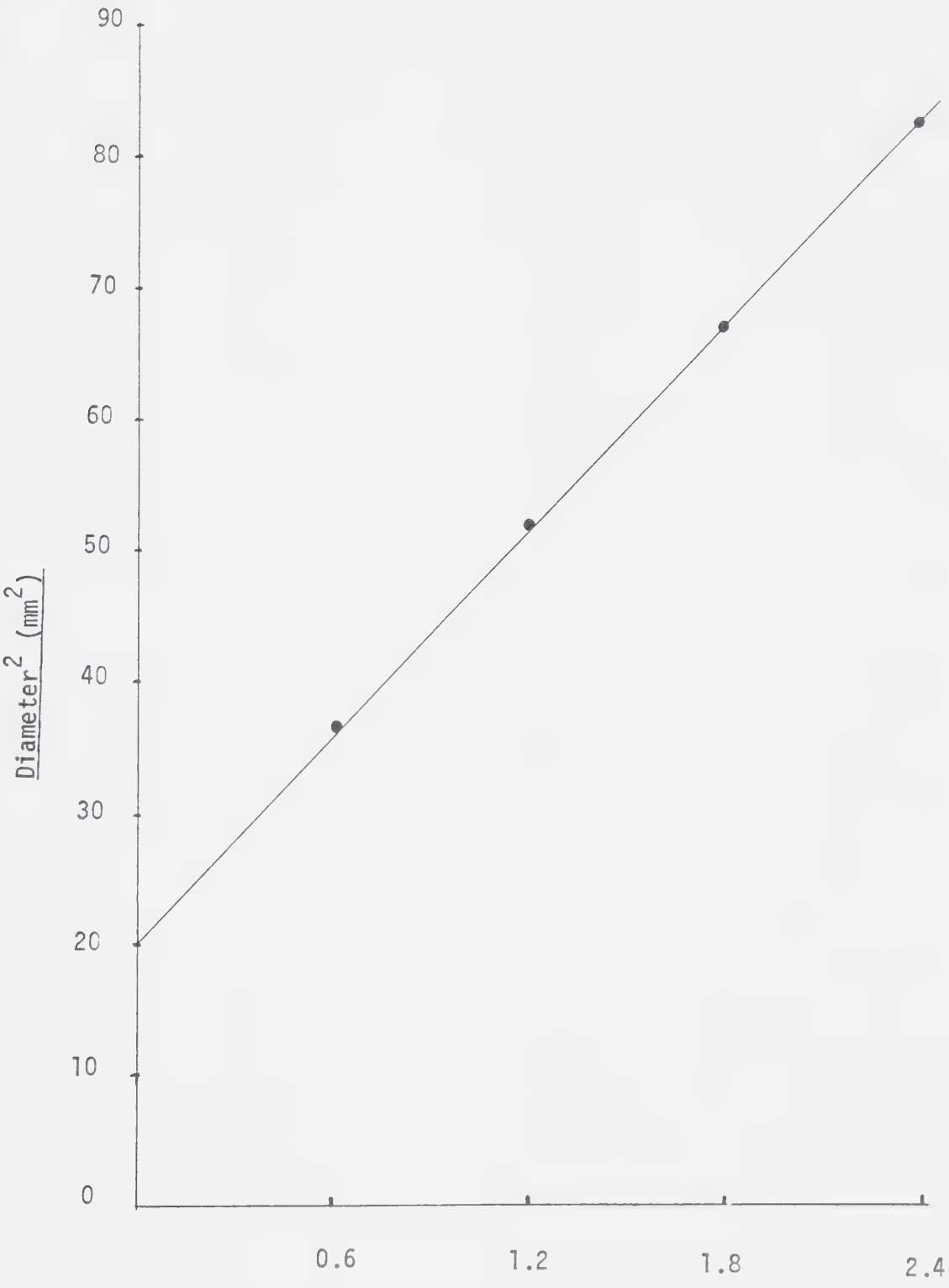


Fig. 2.7

STANDARD CURVE FOR THE DETERMINATION OF RETINOL-BINDING PROTEIN

2.3.9 Statistical Analyses

Means, standard deviations and standard error of the means were determined for patients and controls, and for males and females within each group. One-way analysis of variance was used to determine the significant differences between the means of the different groups for each of the variables. The Student-t test was used to determine if there was a significant difference between the means of any two groups. A pairwise t-test was used to identify any significant change between the first and second samples for patients who had serial samples. Pearson Correlation Coefficients (r) relating the variables to each other were determined. The proportion of survival was derived from the Life-table using the Statistical Package for the Social Sciences (Nie et al., 1975).

Chapter 3

RESULTS

The results are presented in several sub-sections : a) controls, the effect of age and sex on plasma vitamin A and its related factors; b) controls versus patients, with regard to the biochemical parameters such as vitamin A and RBP, cholesterol and cortisol, protein, albumin and globulin; c) patients, those who remained disease-free versus those who had a subsequent recurrence, the effect of site of tumor and the effect of time lapse in between blood sample collection after surgery on vitamin A and its associated factors.

3.1 Controls

The age- and sex-specific changes in plasma vitamin A and the factors involved in its metabolism (i.e., RBP, cortisol and cholesterol) in the control subjects (34 males + 31 females) are shown in Table 3.1. Both male and female subjects were divided into two groups – less than 50 years of age and greater than 50. The rationale for choosing these two age groups was that during the menopausal age, the hormonal differences may have an influence on the biochemical indices which may be different from those of the pre-menopausal age. Statistical analysis (Student t-test) revealed that plasma vitamin A values were not significantly different between the two sexes in either of the age groups (<50, $p=0.93$; >50, $p=0.59$; all ages, $p=0.86$).

TABLE 3.1 EFFECT OF AGE AND SEX ON PLASMA VITAMIN A AND ITS RELATED FACTORS IN CONTROL SUBJECTS.

AGE GROUPS	VITAMIN A (mcg/dl)	CHOLESTEROL (mg/dl)	CORTISOL (mcg/dl)	RETINOL-BINDING PROTEIN (mg/dl)
<50				
MALES (n=18)	76.1 ± 6.5	215.9 ± 12.8	8.5 ± 0.9	6.2 ± 0.5
FEMALES (n=21)	63.9 ± 4.5	223.7 ± 11.8	7.5 ± 0.8	4.1 ± 0.4
SIGNIFICANCE	N.S.	N.S.	N.S.	<0.01
>50				
MALES (n=16)	60.5 ± 7.0	255.8 ± 13.3	9.0 ± 1.0	7.3 ± 0.6
FEMALES (n=10)	58.1 ± 7.7	210.7 ± 16.8	7.3 ± 1.0	5.6 ±0.7
SIGNIFICANCE	N.S.	[illegible]	N.S.	0.05
All Ages				
MALES (n=34)	68.8 ± 4.9	234.7 ± 9.7	8.8 ± 0.6	6.8 ± 0.4
FEMALES (n=31)	62.0 ± 3.9	219.5 ± 9.8	7.5 ± 0.7	4.6 ± 0.4
SIGNIFICANCE	N.S.	N.S.	N.S.	<0.001

Each value represents the mean ± SEM for the number of subjects shown in parenthesis.
N.S. -Not significant

Plasma cortisol had the same trend as vitamin A. Like vitamin A and cortisol, the plasma cholesterol level was not subjected to sex variation before 50 years of age, however, the sex variation was noted in those who were more than 50 years old. The male subjects appeared to have a significantly higher cholesterol level than that of the females. It is interesting to note that the RBP values were found to be consistently and significantly higher in male than in female subjects in both age groups. The difference being more marked in the younger age group.

Among all the biochemical indices measured, only plasma RBP indicated a significant sex difference when both age groups were combined.

3.2 Controls VS Patients

3.2.1 Plasma Vitamin A And RBP In Control Subjects And Postoperative Colorectal Cancer Patients

Since the plasma values of the biochemical indices for the control subjects listed in Table 3.1 were not found to be subjected to age and sex variations, with the exception of RBP, subjects were combined together irrespective of age and sex, and used as "controls" for the cancer patients. Table 3.2 shows the mean differences in plasma vitamin A and RBP between control subjects and the postoperative colorectal cancer patients who appeared to be disease-free. Both groups (B2 & C) of disease-free cancer

patients were found to be associated with not only subnormal plasma vitamin A levels ($p<0.001$), but also lower RBP levels ($P<0.01$) than the control subjects. Relative to the magnitude of its mean value, vitamin A was substantially lower than RBP in both groups (B2 & C) of patients. But RBP values were more negative in the C group than the B2 group. Statistical difference of the RBP levels between control subjects and the patients with 'C' type colorectal cancer was found to be more marked ($p<0.001$) than the difference between controls and the patients with 'B2' type cancer.

3.2.2 Plasma Cholesterol And Cortisol Levels In Control Subjects And Postoperative Colorectal Cancer Patients

Cholesterol and cortisol differences between controls and patients are shown in Table 3.3. Dukes' B2 patients had a mean cholesterol 38.3 mg/dl higher than the controls ($p<0.05$) while the difference between the Dukes' C patients and the controls was not significant ($p=0.10$). Plasma cortisol levels were not significantly different between the controls and the patients.

3.2.3 Plasma Protein, Albumin And Globulin Levels In Control Subjects And Postoperative Colorectal Cancer Patients

The plasma concentrations of total protein in the control subjects were significantly higher ($p<0.001$) than those of the cancer patients (Table 3.4). The albumin/globulin ratio, however, remained unaffected.

TABLE 3.2 PLASMA VITAMIN A AND RBP LEVELS IN CONTROL SUBJECTS AND POSTOPERATIVE COLORECTAL CANCER PATIENTS.

GROUPS	VITAMIN A (mcg/dl)	RETINOL-BINDING PROTEIN (mg/dl)
CONTROLS (n=65)	65.3 ± 3.2	5.7 ± 0.3
COLORECTAL B2 (n=66)	43.5 ± 1.8[1]	5.0 ± 0.3[3]
COLORECTAL C (n=37)	43.1 ± 2.9[1]	3.8 ± 0.4[1]
COLORECTAL B2 + C (n=103)	43.4 ± 1.6[1]	4.6 ± 0.3[2]

Each value is the mean ± SEM for the number of subjects shown in parenthesis.
[1] Significantly different from the controls, $p<0.001$
[2] Significantly different from the controls, $p<0.01$
[3] Significant difference betweeen colorectal B2 and C patients, $p=0.01$.

TABLE 3.3 PLASMA CHOLESTEROL AND CORTISOL LEVELS IN CONTROL SUBJECTS AND POSTOPERATIVE COLORECTAL CANCER PATIENTS.

GROUPS	CHOLESTEROL (mg/dl)	CORTISOSL (mcg/dl)
CONTROLS (n=65)	227.5 ± 6.4	8.1 ± 0.5
COLORECTAL B2 (n=66)	265.8 ± 6.4[1]	8.4 ± 0.5
COLORECTAL C (n=37)	248.3 ± 8.5	9.4 ± 0.6
COLORECTAL B2 + C (n=103)	259.5 ± 4.8[1]	8.8 ± 0.4

Each value is the mean ± SEM for the number of subjects shown in parenthesis.
[1] Significantly different from the controls, $p<0.05$.

TABLE 3.4 PLASMA PROTEIN, ALBUMIN AND GLOBULIN LEVELS IN CONTROL SUBJECTS AND POSTOPERATIVE COLORECTAL CANCER PATIENTS.

GROUPS	PROTEIN (g/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)	A/G RATIO*
CONTROLS (n=65)	7.3 ± 0.1	4.6 ± 0.1	2.8 ± 0.1	1.8 ± 0.1
COLORECTAL B2 (n=66)	6.7 ± 0.1[1]	4.1 ± 0.1[1]	2.4 ± 0.1[2]	1.7 ± 0.1
COLORECTAL C (n=37)	6.5 ± 0.2[1]	3.9 ± 0.1[1]	2.6 ± 0.1	1.7 ± 0.2
COLORECTAL B2 + C (n=103)	6.4 ± 0.1[1]	3.9 ± 0.1[1]	2.5 ± 0.1[3]	1.7 ± 0.1

*Albumin/Globulin ratio.
Each value is the mean ± SEM for the number of subjects shown in parenthesis.
[1] Significantly different from the controls, $p<0.001$.
[2] Significantly different from the controls, $p<0.01$.
[3] Significantly different from the controls, $p<0.05$.

3.3 Patients

3.3.1 Distribution Of Tumor Sites In The Postoperative Colorectal Cancer Patients

Table 3.5 shows the distribution of tumor sites in the patients before surgery. Tumors were located throughout the large bowel. Twelve and six-tenths percent (13/103 : 9 from B2, 4 from C) were in the cecum; 14.5 percent (15/103 : 11 from B2, 4 from C) in the ascending colon; 8.7 percent (9/103 :7 from B2, 2 from C) in the descending colon; 4.9 percent (5/103 : 3 from B2, 2 from C) in the transverse colon; 9.7 percent (10/103 : 6 from B2, 4 from C) in the flexures; 31.1 percent (32/103 : 16 from B2, 16 from C) in the recto-sigmoid; and 18.5 percent (19/103 : 14 from B2, 5 from C) from the rectum.

Due to the small number of cancer sites, subjects were grouped together in the following order : cecum, ascending and descending colon, hepatic and splenic flexures were grouped under colon; sigmoid and recto-sigmoid were grouped under recto-sigmoid; and rectum by itself as a group.

3.3.2 Effects Of Tumor Sites On Plasma Vitamin A And Its Related Factors

Table 3.6 shows that the differences between the cancer sites for all the measured biochemical indices were not significant (vitamin A, $p=0.81$; RBP, $p=0.43$; cholesterol, $p=0.61$; cortisol, $p=0.24$; albumin, $p=0.29$; globulin, $p=0.24$)

TABLE 3.5 DISTRIBUTION OF TUMOR SITES IN THE CANCER PATIENTS BEFORE SURGERY.

SITE OF TUMOR	DUKES' B2		DUKES' C		DUKES' B2 + C	
	n	%	n	%	n	%
CECUM	9	13.6	4	10.8	13	12.6
ASCENDING COLON	11	16.7	4	10.8	15	14.5
DESCENDING COLON	7	10.6	2	5.4	9	8.7
TRANSVERSE COLON	3	4.6	2	5.4	5	4.9
FLEXURES	6	9.1	4	10.8	10	9.7
RECTO-SIGMOID	16	24.2	16	43.3	32	31.1
RECTUM	14	21.2	5	13.5	19	18.5
TOTAL	66	100	37	100	103	100

as determined by one-way analysis of variance.

3.3.3 Effects of Time In Blood Sample Collection Following Surgery On Plasma Vitamin A And Its Related Factors In Cancer Patients

Patients were grouped together by period interval from the date of surgery to the date of blood sample collection. (Table 3.7) The shortest interval (< 2 months) was associated with the lowest average mean of plasma vitamin A level, while during the period of 6 - 12 months following surgery, the average mean values of plasma vitamin A, RBP, cholesterol and cortisol were found to be at their maximum levels. Analysis of variance, however, revealed no overall significant difference in these biochemical indices including albumin and globulin between the various intervals of time (vitamin A, $p=0.45$; RBP, $p=0.43$; cholesterol, $p=0.23$; cortisol, $p=0.13$; protein, $p=0.14$; albumin, $p=0.55$; globulin, $p=0.07$).

3.3.4 Follow-up Studies On Patients With Serial Samples

Of the 103 patients, 40 had serial samples taken at two different periods of time. The time interval between the first and second samples ranged from less than one month to four months with an average time difference of 2.6 months. The results are presented in Table 3.8. No significant differences in plasma concentrations of vitamin A, RBP, cholesterol, cortisol, albumin and globulin were observed

TABLE 3.6 EFFECTS OF TUMOR SITES ON PLASMA LEVELS OF VITAMIN A, RBP, CHOLESTEROL, CORTISOL AND PROTEINS.

TUMOR SITES & DUKE'S STAGE	VITAMIN A (mcg/dl)	RBP (mg/dl)	CHOLESTEROL (mg/dl)	CORTISOL (mcg/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)
COLON*						
B2 (n=36)	42.0 ± 2.5	4.9 ± 0.4	265.8 ± 10.8	8.9 ± 0.8	3.7 ± 0.2	2.4 ± 0.1
C (n=16)	40.1 ± 4.0	4.0 ± 0.6	236.4 ± 12.8	8.0 ± 0.8	4.2 ± 0.3	2.3 ± 0.2
RECTO-SIGMOID						
B2 (n=16)	44.0 ± 3.8	5.3 ± 0.7	263.6 ± 7.7	7.6 ± 0.8	4.1 ± 0.2	2.7 ± 0.2
C (n=16)	45.6 ± 4.8	3.8 ± 0.5	259.4 ± 13.9	10.7 ± 1.4	3.7 ± 0.2	2.7 ± 0.1
RECTUM						
B2 (n=14)	46.3 ± 3.7	5.1 ± 0.5	268.2 ± 11.6	8.3 ± 0.9	4.1 ± 0.2	2.3 ± 0.2
C (n=5)	46.1 ± 8.8	3.3 ± 0.8	250.8 ± 10.1	9.6 ± 1.3	4.0 ± 0.4	2.8 ± 0.4
ANOVA SIGNI-FICANCE	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Each value is the mean ± SEM for the number of subjects shown in parenthesis.
*Includes cecum, ascending and descending colons, transverse colon, hepatic and splenic flexures.
ANOVA=analysis of variance.
N.S.=Not significant.

TABLE 3.7 EFFECT OF TIME LAPSE IN BETWEEN BLOOD SAMPLE COLLECTION AND SURGERY ON PLASMA VITAMIN A AND ITS RELATED FACTORS IN COLORECTAL CANCER PATIENTS.

TIME INTERVAL (MONTHS)	NO. OF PATIENTS (n=103)	VITAMIN A (mcg/dl)	RBP (mg/dl)	CHOLESTEROL (mg/dl)	CORTISOL (mcg/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)
<2	25	38.3 ± 2.6	4.1 ± 0.5	253.2 ± 8.6	9.8 ± 0.7	3.8 ± 0.1	2.7 ± 0.1
2 - 6	9	45.2 ± 7.3	3.7 ± 0.7	225.2 ± 18.3	9.3 ± 2.0	4.0 ± 0.4	2.7 ± 0.2
6 - 12	12	45.6 ± 4.9	5.9 ± 0.7	293.3 ± 15.4	10.4 ± 0.9	4.0 ± 0.2	2.1 ± 0.1
12 - 24	27	45.2 ± 2.8	4.6 ± 0.4	261.7 ± 8.9	7.5 ± 0.4	4.0 ± 0.2	2.5 ± 0.1
>24	30	44.4 ± 3.1	4.7 ± 0.4	259.5 ± 8.4	8.4 ± 0.8	3.8 ± 0.2	2.4 ± 0.1
ANOVA SIGNIFICANCE	---	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Each value is the mean ± SEM for the number of patients.
ANOVA=analysis of variance.
N.S =Not significant.

between the two periods of time. Although in the second sample, patients showed higher mean values of vitamin A and cholesterol (mean difference=7.5 mcg/dl and 6.9 mg/dl, respectively), the plasma RBP, cortisol and albumin levels appeared to be lower than those of the first sample, while the plasma globulin levels remained identical at both periods.

3.3.5 Plasma Vitamin A And Its Related Factors By Disease Status

In follow-up studies to date, of the 103 apparently disease-free colorectal cancer patients, a total of 12 subjects subsequently had a recurrence of the disease. Table 3.9 shows that patients with a recurrence had significantly lower plasma vitamin A levels than those who remained disease-free ($p=0.05$). The patients with subsequent cancer recurrence also showed lower RBP levels than those who remained disease-free. Though the difference was not found to be statistically significant, this may be due to the small sample size of the patients with recurrence.

Plasma cholesterol tended to be lower in recurrent than disease-free cases while cortisol revealed an opposite trend, those with cancer recurrence had higher cortisol values than those who remained disease-free. However, the differences were not statistically significant. Plasma protein, albumin and globulin levels were similar between

TABLE 3.8 PLASMA VITAMIN A, RBP, CHOLESTEROL, CORTISOL, ALBUMIN AND GLOBULIN LEVELS IN PATIENTS WITH SERIAL SAMPLES.

SAMPLES*	VITAMIN A (mcg/dl)	RBP (mg/dl)	CHOLESTEROL (mg/dl)	CORTISOL (mcg/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)
SAMPLE 1 (n=40)	42.4 ± 2.7	4.9 ± 0.4	255.1 ± 8.6	8.7 ± 0.6	3.9 ± 0.1	2.4 ± 0.1
SAMPLE 2 (n=40)	48.9 ± 2.9	4.3 ± 0.3	262.0 ± 9.4	8.1 ± 0.7	3.7 ± 0.1	2.4 ± 0.1
T-TEST 2-TAIL PROBABILITY	0.11 N.S.	0.24 N.S.	0.59 N.S.	0.47 N.S.	0.32 N.S	0.86 N.S.

Each value is the mean ± SEM for the number of subjects shown in parenthesis.
*Mean time interval between the first and second samples=2.6 months, time range=0.75 - 4.00 months.
N.S.=Not significant

TABLE 3.9 PLASMA VITAMIN A AND ITS RELATED FACTORS IN PATIENTS REMAINING DISEASE-FREE AND IN PATIENTS WITH SUBSEQUENT RECURRENCE FOLLOWING SURGERY.

GROUPS	VITAMIN A (mcg/dl)	RBP (mg/dl)	CHOLESTEROL (mg/dl)	CORTISOL (mcg/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)
DISEASE-FREE (n=91)	44.5 ± 1.6	4.6 ± 0.3	261.0 ± 5.1	8.6 ± 0.4	3.8 ± 0.3	2.5 ± 0.1
RECURRENCE (n=12)	35.1 ± 5.2	3.7 ± 0.4	239.8 ± 16.1	10.1 ± 1.1	4.1 ± 0.3	2.4 ± 0.2
SIGNIFICANCE	0.05	N.S.	N.S.	N.S.	N.S.	N.S.

N.S.=Not significant.

the two groups.

When the subsequent recurrent cases were removed from the total of 103 patients who were thought to be free from colorectal cancer following surgery, the results of the comparison of the plasma levels of various biochemical indices related to vitamin A of these remaining disease-free patients with those of the control subjects are shown in Table 3.10. The patterns for the various parameters remained the same as those shown in Table 3.2, 3.3 and 3.4 when the recurrent cases were included. Analysis of variance indicated that all biochemical measurements (except cortisol) were significantly different between control subjects and the apparently disease-free postoperative colorectal cancer patients. Thus, the plasma vitamin A and RBP were lower in patients than in controls ($p<0.001$; $p=0.001$) while plasma cholesterol was higher in patients than in controls ($p<0.05$). Plasma protein, albumin and globulin were consistently lower ($p<0.001$) in patients than in controls.

It was interesting to note that both plasma concentrations of vitamin A and RBP were very low in the two patients who died of the disease during this study. The plasma vitamin A values of these two patients were found to be only 19.32 mcg/dl and 18.86 mcg/dl, and plasma RBP values were 2.40 mg/dl and 1.68 mg/dl, respectively (Table 3.11).

TABLE 3.10 PLASMA VITAMIN A AND ITS RELATED FACTORS IN CONTROL SUBJECTS AND APPARENTLY DISEASE-FREE POSTOPERATIVE COLORECTAL CANCER PATIENTS.

GROUPS	VITAMIN A (mcg/dl)	RBP (mg/dl)	CHOLESTEROL (mg/dl)	CORTISOL (mcg/dl)	PROTEIN (g/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)
CONTROLS (65)	65.3 ± 3.2	5.7 ± 0.3	227.5 ± 6.4	8.1 ± 0.5	7.3 ± 0.1	4.6 ± 0.1	2.8 ± 0.1
COLORECTAL B2 (60)	44.3 ± 1.9[1]	5.1 ± 0.3	267.5 ± 6.2[3]	8.3 ± 0.5	6.3 ± 0.1[1]	3.9 ± 0.1[1]	2.4 ± 0.1[3]
COLORECTAL C (31)	44.7 ± 2.8[1]	3.6 ± 0.4[1]	251.5 ± 8.2[3]	9.2 ± 0.8	6.4 ± 0.1[1]	3.8 ± 0.2[1]	2.6 ± 0.1
COLORECTAL B2 + C (91)	44.5 ± 1.6[1]	4.6 ± 0.3[2]	262.1 ± 5.0[3]	8.6 ± 0.4	6.4 ±0.1[1]	3.9 ± 0.1[1]	2.5 ± 0.1[3]
ANOVA SIGNIFICANCE	<0.001	0.001	<0.05	N.S	<0.001	<0.001	<0.001

Each value is the mean ± SEM for the number of subjects shown in parenthesis.
[1]Significantly different from the controls, $p<0.001$.
[2]Significantly different from the controls, $p<0.01$.
[3]Significantly different from the controls, $p<0.05$.
ANOVA=analysis of variance.
N.S.=Not significant.

TABLE 3.11 CLINICAL AND PATHOLOGICAL CHARACTERISTICS OF PATIENTS WHO DIED OF THE DISEASE DURING THE STUDY.

CODE*	AGE	SEX	STAGE OF DIGNOSIS	PRIMARY SITE	SURVIVAL FOLLOWING SURGERY (MONTHS)	VITAMIN A (mcg/dl)	RBP (mg/dl)	CHOLE-STEROL (mg/dl)	CORTISOL (mcg/dl)	ALBUMIN (g/dl)	GLOBULIN (g/dl)
2001	68	M	B2	SIGMOID COLON**	5	19.32	2.40	250	10.40	3.58	3.62
2104	78	M	C	ASCEND. COLON**	8	18.86	1.68	248	11.52	3.67	2.28

*Patient's code no.
**Metastasis to liver.

3.3.6 Disease-free Survival

Fig 3.1 shows the survival rate for all apparently disease-free patients included in this study. The survival rate was calculated from the date of surgery to either the date of cancer recurrence or death. Currently, 84 percent of the B2 and 64 percent of the C patients have survived. The mean disease-free survival for Dukes' B2 and Dukes' C patients was 41.1 months and 24.8 months, respectively. Pairwise comparison using the non-parametric test indicated that the disease-free survival rates between the two groups are significantly different ($p=0.05$).

3.4 Relationship Between Plasma Vitamin A, RBP, Cholesterol And Cortisol

The relationships between plasma vitamin A and RBP, cholesterol and cortisol are shown in Figures 3.2, 3.3. and 3.4.

Plasma vitamin A was significantly correlated with RBP for the control subjects ($r=0.06$, $p=<0.05$) but not for the postoperative colorectal cancer patients ($r=0.18$) (Fig. 3.2). However, the plasma vitamin A concentrations did not show any significant correlations with either cholesterol or cortisol; this pattern was consistent for both control subjects and patients (Fig. 3.3 & Fig. 3.4). Nonetheless, the control subjects tended to show a negative relationship between plasma vitamin A and cholesterol ($r=-0.13$) while patients indicated a negative relationship between plasma

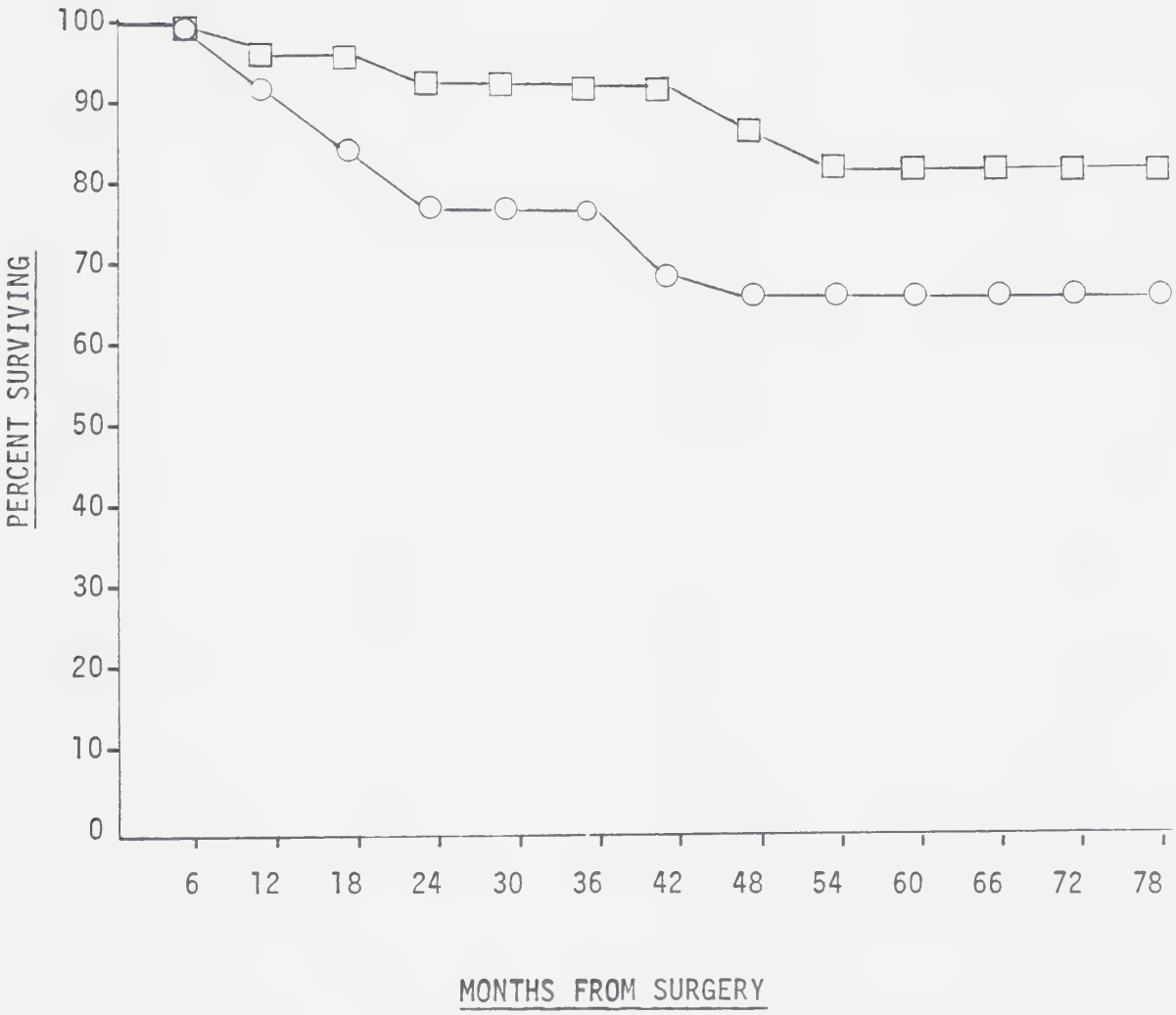
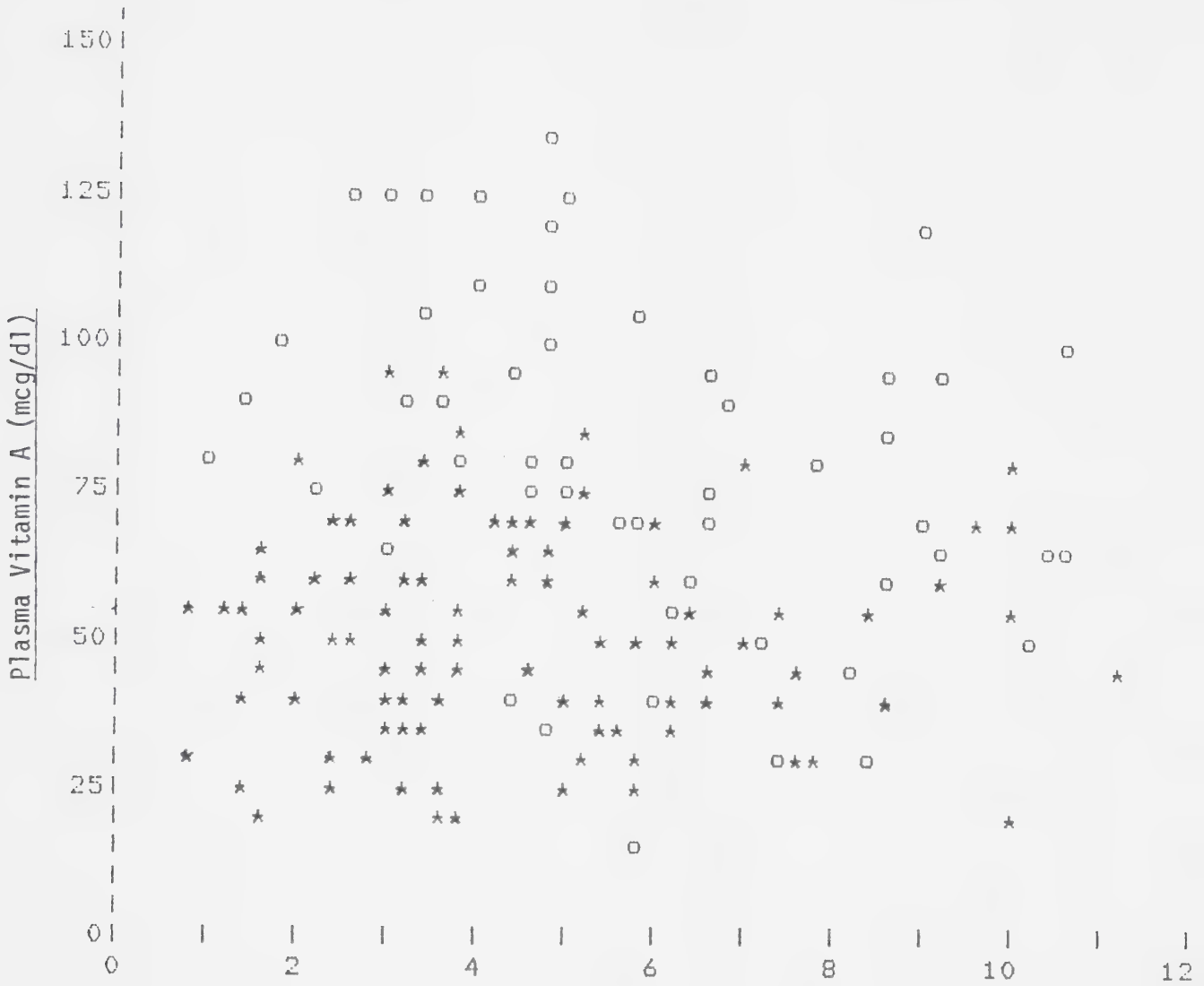


Fig. 3.1 DISEASE-FREE SURVIVAL BY DUKES' STAGE

vitamin A and cortisol (r=-0.21).



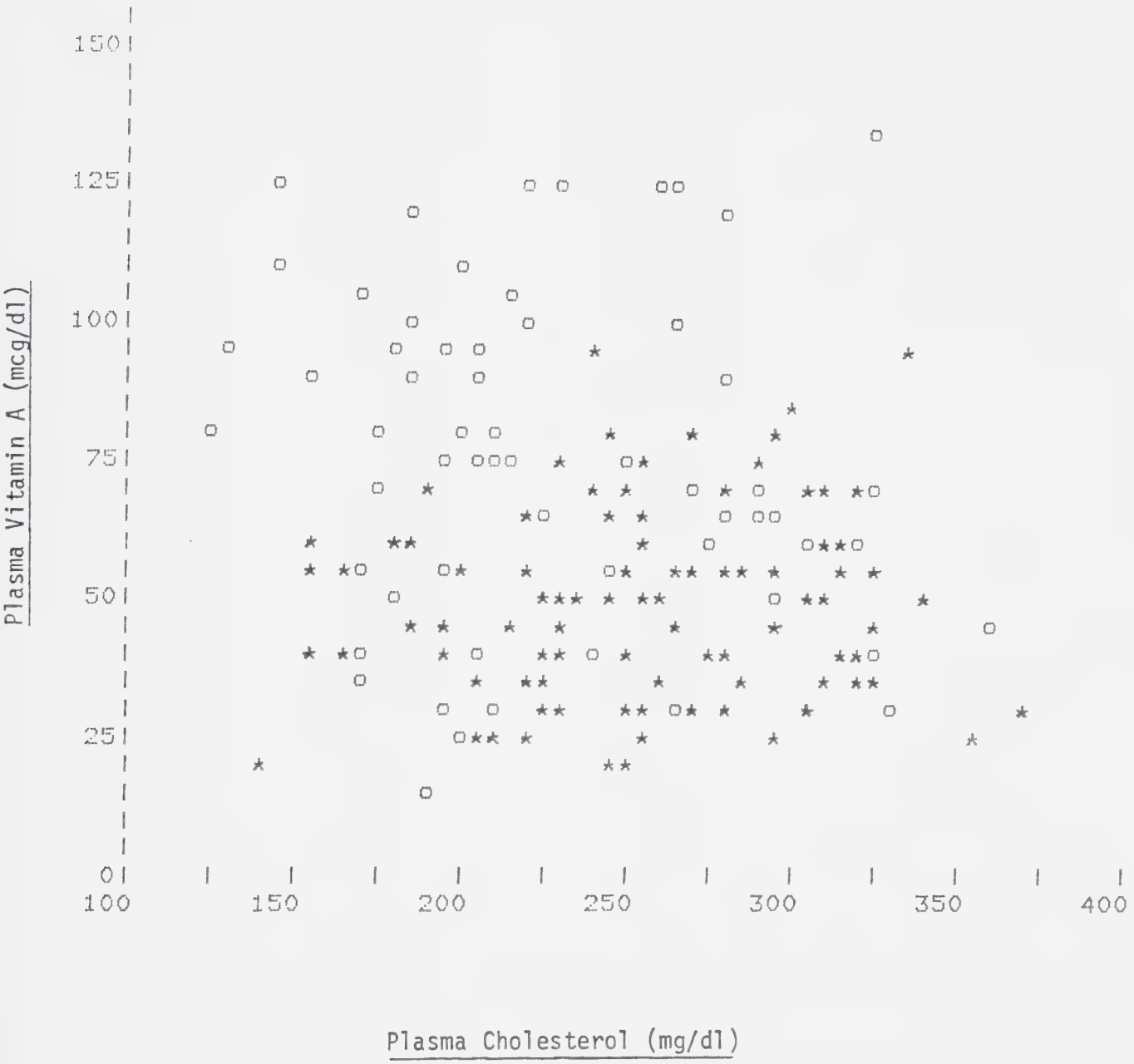
o = Controls (n=65)

★ = Postoperative colorectal cancer patients (n=103)

For controls : r=0.06, p=0.05
y=71.02 + 0.77x

For patients : r=0.18, p=N.S.

Fig. 3.2 RELATIONSHIP BETWEEN PLASMA VITAMIN A AND RETINOL-BINDING PROTEIN IN CONTROL SUBJECTS AND POSTOPERATIVE COLORECTAL CANCER PATIENTS.

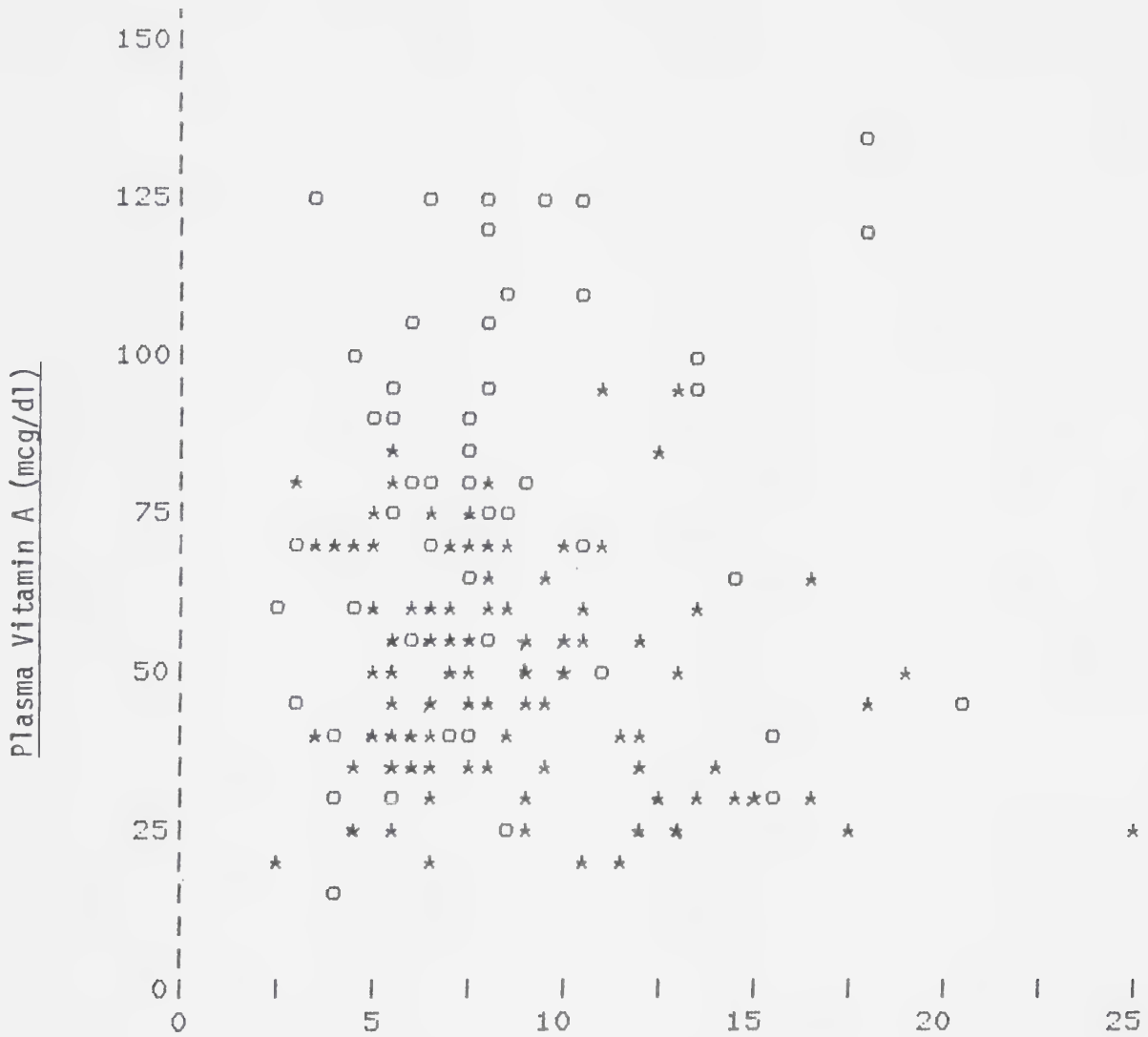


o = Controls (n=65)

* = Postoperative colorectal cancer patients (n=103)

For controls : r=-0.13, p=N.S.
For patients : r=0.13, p=N.S.

Fig. 3.3 RELATIONSHIP BETWEEN PLASMA VITAMIN A AND CHOLESTEROL IN CONTROL SUBJECTS AND POSTOPERATIVE COLORECTAL CANCER PATIENTS.



o = Controls (n=65)

* = Postoperative colorectal cancer patients (n=103)

For controls : r=0.34, p=N.S.
For patients : r=-0.21, p=N.S.

Fig. 3.4 RELATIONSHIP BETWEEN PLASMA VITAMIN A AND CORTISOL IN CONTROL SUBJECTS AND POSTOPERATIVE COLORECTAL CANCER PATIENTS.

Chapter 4

Discussion

It has long been known that vitaman A is required for the normal histology of epithelial cells. A recent concern has been the relationship between vitamin A deficiency and cancer of epithelial origin. Experimental studies have shown that vitamin A deficiency increases susceptibility to chemical carcinogenesis which can be reversed by giving vitamin A supplementation. Both biochemical and dietary studies have provided evidence to support a link between vitamin A deficiency and cancers of epithelial cell origin in man . These studies present a consistent picture as to vitamin A deficiency being a possible risk factor for cancers of the lung, bronchus and gastrointestinal tract, in particular. However, the low plasma vitamin A levels observed in these cancer patients may be largely due to a prolonged poor dietary intake and absorption in the presence of the slow growing tumor.

The present study was undertaken to investigate the biochemical status of vitamin A in colorectal cancer patients who had undergone surgery and were considered to be apparently free from the presence of tumor. One hundred and three subjects were studied which was considerably higher than the number of subjects studied in most of the other reported studies. The circulating levels of vitamin A of these patients were measured in order to provide biochemical evidence of their vitamin A status. In addition, the

results were compared with a group of apparently healthy subjects. These healthy subjects were not used as true controls but were used to merely provide normal values of vitamin A using the same method employed to determine plasma vitamin A levels in the cancer patients. Such a measure was considered important as reported results for plasma vitamin A in cancer patients were based on a number of methods used in different laboratories, thus resulting in a wide variation in reported normal ranges. The study by Wald et al. (1980) showed that 86 men with lung cancer compared to 172 controls, matched for age and smoking history had a significantly lower plasma retinol level (56 mcg/dl vs 69 mcg/dl for controls, $p<0.005$). In a second study, Kark et al. (1981) showed that among 85 cancer patients compared to 174 age, race and sex-matched controls, the patients had significantly lower plasma retinol levels (41 mcg/dl vs 47 mcg/dl in controls, $p<0.003$). Although both of these studies were done with meticulous care and good methodology, the mean plasma retinol levels were quite different. In the Wald study, the controls had 69 mcg/dl of retinol, whereas in the Kark study, the controls had only 47 mcg/dl. The vitamin A assays used were different. Wald et al. used high-performance liquid chromatography (HPLC) whereas Kark et al. used alumina chromatogrphy followed by a fluorimetric method for estimation of retinol.

Results in the present study indicated that the 103 postoperative colorectal cancer patients, though they were

seemingly free of the disease, had subnormal levels of vitamin A when compared with the apparently healthy subjects (43.4 mcg/dl vs 65.3 mcg/dl) (Table 3.2). This finding is consistent irrespective of either the stage of the disease or the site of the tumor (Table 3.6).

Subnormal levels of plasma vitamin A, measured as retinol have been observed in cancers of the lung (Atukorala et al., 1979; Basu et al., 1976; Basu et al., 1982); oropharynx (Ibrahim et al., 1979) and GI tract (Abels et al., 1941). In these studies, blood samples were taken from people who still had a tumor. It is therefore possible that their appetite was depressed due to the presence of the tumor, or the tumor itself may have lowered retinol levels due to its increased requirement. Moreover, the treatment of the disease by either chemotherapy or radiotherapy may have resulted in a taste aberration, thus depressing food intake and causing subsequent nutritional problems (DeWys, 1974; DeWys, 1975).

It is noteworthy to point out that the patients in the present study had already undergone resection of their carcinomas and were believed to be free of neoplastic disease following surgery when the blood samples were collected. In addition, these patients were not undergoing any kind of therapy and yet they were found to have subnormal circulating levels of retinol. This was true not only when the blood samples were collected soon after surgery but also in the subsequent samples collected 3/4 to

4 months later (Table 3.8). The low circulating vitamin A levels may have resuslted for other reasons, some of which will be discussed below.

4.1 Cortisol

Corticosteroids have been reported to be antagonistic to vitamin A by favouring its elimination from the body. Thus, a negative association between vitamin A and corticosteroids have been reported in animal studies (Clark & Colburn, 1955; Atukorala et al., 1981) in which administration of cortisone to rats over a long period appeared to depress vitamin A concentration in the plasma, liver, adrenal glands, thymus and kidney. The affect was greatest on the thymus, in which marked thymic shrinkage was accompanied by a rapid depletion of vitamin A. Physical stress was also shown to precipitate frank vitamin A deficiency in rats on a marginal intake of the vitamin (Seifter et al., 1973), possibly as a result of stress-induced secretion of adrenocorticotrophic hormones (ACTH). Human studies have also indicated that epithelial cancer of the breast (Desphande et al., 1969), prostate (Doe et al., 1969) and lung (Lichter & Sirett, 1968) are associated with elevated plasma levels of corticosteroids. It is possible that there is an increased requirement for vitamin A in the presence of an elevation of corticosteroids. However, in the present study, cortisol levels remained unaffected while vitamin A levels were

subnormal, therefore, it is unlikely that corticosteroids could be responsible for the low vitamin A levels in the disease-free postoperative colorectal cancer patients. This finding is further substantiated by the fact that plasma concentration of cholesterol, which is a precursor of cortisol, also remained unaffected.

4.2 Cholesterol

Vitamin A or its precursor is absorbed into the intestinal mucosal cells, along with the products of digestion of dietary fat, and re-esterified within the intestinal mucosa. The retinyl esters so formed are circulated as constituents of chylomicrons. Thus, low plasma levels of vitamin A might result from the malabsorption of fat. The subnormal levels of plasma vitamin A found in the cancer patients may be due to impaired fat absorption since the efficient intestinal absorption of vitamin A is dependent on a structurally intact intestinal tract, a properly functioning pancreas and an unimpaired flow of bile. The radical surgery used in the treatment of the malignancies of the GI tract may leave patients with a reduced capacity to ingest or absorb nutrients (Shils, 1979). However, the physiological and nutritional consequences of surgical resection of the GI tract of cancer have been carefully reviewed by Lawrence (1977) who indicated that, unlike resection of the thoracic esophagus, stomach or pancreas, subtotal and total resection

of the colon is well tolerated from the nutritional standpoint and rarely produces nutritional deficiencies. Therefore, it is unlikely that the lower plasma vitamin A level is due to malabsorption. Furthermore, the plasma level of cholesterol, another fat soluble substance, was found to be higher in the postoperative colorectal cancer patients than in the control subjects. These findings tend to exclude the possiblity of lipid - metabolism being involved in causing subnormal vitamin A levels in these patients.

Since reports in the literature on the relation of plasma cholesterol levels to risk of death from cancer contain contradictory findings, it is noteworthy to point out the results of plasma cholesterol in this study. Several studies have suggested an inverse relationship between plasma cholesterol and colon cancer (Rose et al., 1974, Kark et al., 1980; Williams et al., 1981; Peterson et al., 1981; Kagan et al., 1981; Garcia-Palmieri et al., 1981), while others have found no such association (Dyer et al., 1981; Kozarevic et al., 1981; Thomas et al., 1982).

The present study revealed higher plasma concentration of cholesterol in postoperative colorectal cancer patients than that of the control subjects. However, when the results of those who had a subsequent recurrence (n=12) were compared to those who remained disease-free (n=91), the former group had a lower plasma cholesterol level (239.8 mg/dl vs 261.0 mg/dl). Although the difference was not

found to be statistically significant (probably due to the small number of recurrent cases), the trend is in agreement to those reported by Rose et al. (1974). They pooled the colon cancer deaths from various cardiovascular studies ("Seven Countries"; Framingham; Chicago Gas Company; Whitehall, London; Minnesota businessmen; & Chicago Western Electric company) yielding 90 cases of colon cancer whose plasma cholesterol levels were significantly lower ($p<0.05$) than those of the control subjects. In a more recent study in Honolulu (Kagan et al., 1981), patients with colon cancer were also found to be negatively associated with cholesterol level. Furthermore, a case-control study (Kark et al., 1980), carried out in the Evans County, Georgia, revealed a significantly lower serum cholesterol level in those who subsequently (12 - 14 years later) developed cancer.

In contrast to the above reports, Thomas et al. (1982) found no consistent relationship between baseline cholesterol level and cancer during a follow-up study of 30 male medical students who later developed major cancer over an extended period of 33 years. Dyer et al. (1981) pooled the data from 3 Chicago epidemiological studies and found no significant association between serum cholesterol level and the incidence of lung, colorectal, oral and pancreatic cancers.

It appears, therefore, that the results of the present study showing low plasma cholesterol level in the 12 patients with subsequent recurrence of colorectal cancer

only contribute to the continuing controversy. The reasons for this observation are at present unclear.

This lack of symmetry in the association of cholesterol with cancer, and the lack of correlation between vitamin A with cholesterol require comment. Vitamin A appears to be more independent of cholesterol in its association with cancer than is true of the reverse. This may imply that vitamin A is causally involved in carcinogenesis while cholesterol is simply a concomitant.

4.3 Retinol-binding Protein (RBP)

RBP has long been associated with the metabolism of vitamin A. Vitamin A is mobilized from liver stores and transported in plasma in the form of the lipid alcohol retinol, bound to RBP, which is a single polypeptide chain with a molecular weight close to 20,000. RBP has a single binding site for one molecule of retinol and is present in plasma mainly as the RBP-retinol complex (holo-RBP). In addition, RBP interacts strongly with plasma prealbumin and normally circulates as a 1:1 molar RBP-prealbumin complex.

RBP is responsible for the delivery of retinol from the liver to the extrahepatic sites of action of the vitamin. Evidence is available that this delivery process may involve cell surface receptors for RBP, thus, studies have been reported which suggest that there are specific cell surface receptors for RBP on monkey small intestine mucosal cells (Rask & Peterson, 1976), on bovine pigment epithelial cells

(Heller, 1975; Chen & Heller, 1977), and on chicken testicular cell membranes (Bhat & Cama, 1979). In these studies, retinol appeared to be taken up (from holo-RBP) by the cells without a concomitant uptake of RBP. Thus, RBP appears to deliver retinol to specific surface sites that "recognize" RBP, and to release retinol at these locations. After its delivery, the retinol enters the cell for subsequent metabolism and action. The apo-RBP (i.e. RBP without retinol) does not appear to enter the cell, but returns to the circulation, where it shows a reduced affinity for prealbumin and is selectively filtered by the renal glomeruli.

Results in the present study have shown that, in contrast to plasma cortisol and cholesterol, the circulating RBP levels were significantly lower in the apparently disease-free colorectal cancer patients. Since vitamin A is transported to the target tissues bound to RBP, the low plasma RBP level in parallel with low plasma vitamin A raises the possibility that subnormal levels of vitamin A in the colorectal cancer patients may be the result of a lower concentration of the carrier protein. Low concentrations of both plasma vitamin A and RBP have also been observed by Atukorala (1979) in lung cancer patients, by Basu et al. (1982) in patients with epithelial cancer but not in myeloma patients.

Analyses of data available to date have indicated that subnormal plasma vitamin A levels exist long before the

appearance of tumor. Thus, subnormal levels of vitamin A were observed in subjects who subsequently developed cancer (Wald et al., 1980; Kark et al., 1981). However, RBP levels are not affected until the tumor is present. Thus, in a case-control study by Haines et al. (1982), plasma levels of RBP and vitamin A were measured in samples taken 2 - 7 years before the development of clinically manifested tumors. Results indicated that there was no difference in RBP levels between cases and controls. This specific characteristic of RBP suggests that plasma level of the vitamin A carrier protein could be used as a tumor marker. Such a possibility is further substantiated by the observations made in the present study where colorectal cancer patients with (Dukes' C) or without (Dukes' B2) regional lymph-node metastases were found to be associated with subnormal plasma vitamin A levels, while RBP levels appeared to be below normal only in Dukes' C patients. Furthermore, in follow-up studies to date, besides displaying significantly lower plasma vitamin A values ($p=0.05$), patients who had a subsequent recurrence of the disease also had lower RBP values than those who remained free of apparent cancer (3.7 mg/dl vs 4.6 mg/dl), although the difference between the two groups was not statistically significant. The number of recurrent patients was small and the period of study was short.

The exact mechanism for the decreased level of RBP cannot be explained at this stage. However, a number of factors could be involved, namely : a) malnutrition; b)

decreased synthesis of RBP; c) decreased level of vitamin A in the liver; d) increased utilization of vitamin A.

4.3.1 Malnutrition

Various studies have indicated that depressed vitamin A and RBP levels may be associated with protein deficiency. Low levels of plasma vitamin A and RBP have been observed with low concentrations of total protein and albumin in a group of Egyptian children with protein-calorie malnutrition (Smith et al., 1973a). Supplementation to these children with calories and protein, without concomitant vitamin A therapy, was found to cause a gradual elevation of vitamin A and RBP to normal levels. Although malnutrition is thought to be less common in cancer patients who have undergone radical surgery of the colon and rectum (Lawrence, 1977), the total protein and albumin concentrations in the plasma were found to be significantly lower in the patients included in the present study than those in the healthy subjects ($p<0.001$). It is, therefore, possible that the decreased plasma concentrations of vitamin A and RBP in the colorectal cancer patients were a manifestation of a generalized nutritional deficiency. However, in view of the fact that the albumin and globulin ratio, the plasma cholesterol and cortisol levels in these patients were all found to be normal, the subnormal levels of plasma vitamin A and RBP would less likely be exclusively due to malnutrition.

4.3.2 Decreased Synthesis Of RBP

It has been reported that zinc is involved in the synthesis of RBP. In the presence of zinc deficiency, RBP levels could be expected to be low which could, in turn, affect plasma vitamin A levels. Experimental studies have demonstrated that rats fed a zinc-deficient diet had markedly lower concentrations of plasma RBP and vitamin A than rats fed ad libitum (Smith et al., 1974; Ette et al., 1979). The liver concentration of RBP in zinc-deficient rats was only 55 - 60 percent of that of the ad libitum fed rats. Repletion of the zinc-deficient animals restored the plasma vitamin A to values within the normal range (Brown et al., 1976). These studies suggested that zinc deficiency might interfere with the synthesis of RBP which in turn affects vitamin A mobilization. Indeed, decreased plasma zinc concentraions in parallel with vitamin A and RBP have been observed in various cancer patients (Davies et al., 1968; Morgan, 1970; Atukorala et al., 1979; Basu et al., 1982).

Unfortunately, plasma zinc levels of the patients involved in the present study were not measured because of the unavailability of an atomic absorption spectrophotometry facility. The measurement of this trace element would surely add more light to the present findings.

4.3.3 Decreased Level Of Vitamin A In The Liver

Vitamin A mobilization from the liver is regulated by factors that control the rates of RBP synthesis and secretion. One factor which specifically regulates RBP secretion from liver is the nutritional vitamin A status. Studies in the rat have shown that in the retinol-deficient state, the secretion of RBP from the liver is blocked, resulting in the accumulation of an enlarged pool of apo-RBP (Muto et al., 1972; Smith et al., 1973b). Conversely, repletion of retinol-deficient rats with retinol stimulates the rapid secretion of RBP from the expanded liver pool to the plasma (Smith et al., 1973b).

4.3.4 Increased Utilization Or Requirement Of Vitamin A

It has been suggested by a number of studies that there may be an increased requirement for vitamin A in the presence of a tumor. Thus , both cellular retinol-binding protein (CRBP), which binds retinol, and cellular retinoic acid-binding protein (CRABP), which binds retinoic acid, have been detected in human cancerous tissues of the lung, breast and stomach (Clamon et al., 1981; Lotan et al., 1980; Ong et al., 1975; Ong & Chytil, 1976), but not in adjacent non-malignant tissues. These findings suggest that there may be an increased requirement or utilization of vitamin A in these target cells. If this is so, there may be an extra burden on the liver to synthesize RBP at a faster rate, which could consequently exceed the rate of synthesis of the

protein leading to RBP deficiency. Whether vitamin A requirement is increased in the presence of the tumor cannot be determined by measuring only vitamin A per se, but degradation products such as retinaldehyde and retinoic acid must also be measured. To date, the method for determining retinoic acid is not available. Indeed, further research could be geared towards establishing a satisfactory method by which retinoic acid could be measured.

4.4 Reduced Dietary Intake

Recent literature has pointed out that reduced dietary intake could be responsible for the low plasma vitamin A in cancer patients. Both retrospective and prospective epidemiological dietary studies (Bjelke, 1975; Hirayama, 1979; Mettlin et al., 1979) have suggested that an inverse relation of the RR of human cancer, of epithelial cell origin in particular, and the dietary intake of vitamin A. However, as mentioned earlier (see page 16), the results of these studies must be viewed with caution as they did not take into consideration the ingestion of synthetic vitamin preparations or vitamin A-rich animal foods such as liver. Moreover, there may be some bias in these studies since patients may not be able to recall accurately foods eaten over an extended period of time, prior to the onset of the disease. In addition, feeling ill or knowing they have cancer may cause patients to describe their past diet somewhat differently from how they would have described it

if they had been healthy controls.

No information concerning the usual dietary intake of vitamin A of the patients included in the present study is available since they were sent home after they were resected "for cure" for their carcinomas and were believed to be free of evident disease. Furthermore, there are obvious difficulties in establishing such a relationship since plasma vitamin A levels may remain unaffected even when an individual had been on a vitamin A deficient diet for a prolonged period of time, as was indicated in the Sheffield Study mentioned earlier (see page 10). Therefore, what appears to be necessary is a long term combined epidemiological and biochemical study of a population "at risk" extending over several decades.

4.5 Conclusion

The work presented in this study has pointed to a series of associations that may be of causal importance in the prediction of subsequent recurrence of colorectal cancer in postoperative cancer patients who are considered to be free of the disease.

The patients in this study were found to have biochemical evidence of a vitamin A deficiency as determined by their plasma vitamin A levels. This deficiency was not due to the effect of surgery since a) all blood samples were collected at least 1 1/2 months after surgery, and b) in a follow-up study involving 40 out of 103 patients, vitamin A

levels appeared to be similar to the values obtained in the first sample of these patients. On the basis of these results, the possibility of the dietary factor being involved in lowering the plasma vitamin A concentrations in the postoperative colorectal cancer patients cannot be excluded, however, the low RBP along with low plasma vitamin A levels in these patients certainly indicate that the bioavailability of this vitamin could be impaired. The decreased availability of this vitamin is further exacerbated in those patients who had a recurrence of the disease. Whatever the mechanism may be, the fact that these disease-free postoperative colorectal cancer patients have low circulating vitamin A could justify a supplementation of the vitamin. However, before such a measure is taken, it is of paramount importance to determine the rate of degradation of vitamin A in these patients, since the subnormal vitamin A in the plasma may be a reflection of the degradation imposed by the increased requirement for retinoic acid. Until this is done, these subjects should be given a well-balanced diet containing adequate sources of vitamin A rather than supplementation of large doses of the vitamin. Supplementation with vitamin A in excessive amounts could possibly aggravate the growth of the cancerous tissues, should the need of these tissues for vitamin A is indeed increased.

Nonetheless, the evidence to date (Basu et al., 1983) has indicated that subnormal levels of vitamin A exist long

before the existence of cancer but RBP levels decrease only in the presence of the tumor. On the basis of this evidence, possibly RBP could be used as a tumor marker. Such a hypothesis is further substantiated by the evidence in the present study, as plasma vitamin A was subnormal in colorectal patients with (Dukes' C) or without (Dukes' B2) nodal involvement, while RBP was found to be low only in patients with lymph-node metastases. Furthermore, RBP, in conjunction with vitamin A, was found to be even lower in those who had a recurrence of cancer than in those who remained disease-free.

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